THE ASSOCIATION OF VITAMIN D WITH METABOLIC DISORDERS UNDERLYING TYPE 2 DIABETES

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Graduate Department of Nutritional Sciences
University of Toronto

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Abstract

Emerging evidence suggests that vitamin D may be associated with type 2 diabetes (T2DM), however current data are inconsistent regarding metabolic disorders underlying T2DM. The objectives of this thesis were to investigate the association of vitamin D with the primary pathophysiological disorders of type 2 diabetes: namely insulin resistance (IR) and beta (β)-cell dysfunction, and the metabolic syndrome (MetS).

All studies included individuals participating in the PROspective Metabolism and ISlet cell Evaluation (PROMISE) cohort study, comprising 712 subjects 30 years and older, and at risk of T2DM at baseline. Serum 25-hydroxyvitamin D [25(OH)D] was measured to assess vitamin D nutritional status. Validated oral glucose tolerance test derived indices for IR and β-cell function were calculated.

In the first cross-sectional study, multivariate linear regression analyses indicated a significant inverse association of serum 25(OH)D with IR (β=-0.003, p=0.007) and a significant positive association of 25(OH)D with β-cell function (β=0.004, p=0.03) at the baseline PROMISE clinic visit (n=712). In another cross-sectional study also conducted using data from the baseline PROMISE clinic visit, higher 25(OH)D was found to be
significantly associated with a reduced presence of the MetS after multivariate adjustment (OR=0.76, 95% CI 0.62-0.93). Low serum 25(OH)D was also significantly associated with various MetS components. In light of the findings in the first cross-sectional study, the third study examined prospective associations of baseline 25(OH)D with 3-year follow-up IR and β-cell function (n=489). Although baseline 25(OH)D was not significantly associated with follow-up IR, a significant positive association of baseline 25(OH)D with β-cell function at follow-up was observed (β=0.005, p=0.015). Lastly, in a longitudinal substudy (n=127), seasonal changes in 25(OH)D over 2.5 years did not significantly affect changes in IR and β-cell function.

In conclusion, results indicated that baseline serum 25(OH)D was cross-sectionally related to IR, β-cell function and the MetS, and was prospectively related to β-cell function at the 3-year follow-up. In addition, seasonal changes in 25(OH)D do not adversely affect IR and β-cell function over time. These findings suggest a potential role for higher 25(OH)D levels in reducing diabetes risk, although additional longitudinal studies are warranted.
Acknowledgements

First and foremost, I would like to thank God for all the blessings He’s given me - for my family and friends, for the abilities and the opportunities and also for the strength and guidance.

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<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D or calcidiol</td>
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<tr>
<td>1,25(OH)₂D</td>
<td>1,25-dihydroxyvitamin D₃ or calcitriol</td>
</tr>
<tr>
<td>7-DHC</td>
<td>7-dehydrocholesterol</td>
</tr>
<tr>
<td>AI</td>
<td>Adequate Intake</td>
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<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>DRI</td>
<td>Dietary Reference Intake</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated Average Requirement</td>
</tr>
<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate</td>
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<tr>
<td>FPG</td>
<td>Fasting plasma glucose</td>
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<tr>
<td>FPI</td>
<td>Fasting plasma insulin</td>
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<tr>
<td>IFG</td>
<td>Impaired fasting glucose</td>
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<tr>
<td>HDL-C</td>
<td>High-density lipoprotein cholesterol</td>
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<tr>
<td>HOMA-β</td>
<td>Homeostasis model assessment of beta-cell function</td>
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<tr>
<td>HOMA-IR</td>
<td>Homeostasis model assessment of insulin resistance</td>
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<tr>
<td>IGI</td>
<td>Insulinogenic index</td>
</tr>
<tr>
<td>IGT</td>
<td>Impaired glucose tolerance</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin Resistance</td>
</tr>
<tr>
<td>IS₂OGTT</td>
<td>Insulin Sensitivity Index (from an oral glucose tolerance test)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>ISSI-2</td>
<td>Insulin secretion sensitivity index-2</td>
</tr>
<tr>
<td>IU</td>
<td>International unit</td>
</tr>
<tr>
<td>MetS</td>
<td>Metabolic Syndrome</td>
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<tr>
<td>NAFLD</td>
<td>Non-alcoholic fatty liver disease</td>
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<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>PROMISE</td>
<td>Prospective Metabolism and Islet cell Evaluation cohort</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid Hormone</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
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<tr>
<td>RDA</td>
<td>Recommended Dietary Allowance</td>
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<tr>
<td>RXR</td>
<td>Retinoid X receptor</td>
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<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
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<tr>
<td>TG</td>
<td>Triglyceride</td>
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<tr>
<td>UL</td>
<td>Tolerable Upper Intake Level</td>
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<tr>
<td>UV</td>
<td>Ultraviolet</td>
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<tr>
<td>UVB</td>
<td>Ultraviolet B radiation</td>
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<tr>
<td>VDBP</td>
<td>Vitamin D binding protein</td>
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<tr>
<td>VDR</td>
<td>Vitamin D receptor</td>
</tr>
<tr>
<td>VDRE</td>
<td>Vitamin D response element</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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Statement of Contributions

In this thesis work, I identified and refined the research questions and objectives; conducted the literature review; performed the assays for 25(OH)D in the baseline blood samples in Dr. Vieth's lab; coordinated the 25(OH)D measurements in the follow-up blood samples; organized substudy 6-year clinic visits with PROMISE study coordinators; worked in the Mount Sinai Diabetes unit with PROMISE study nurses and assisted in all substudy 6-year clinic visits (administering questionnaires, preparing blood for the lab); cleaned, merged, and performed quality control assessments in the datasets for all studies; conducted all analyses independently; wrote abstracts and manuscripts (including 3 published papers), and presented research findings at national and international conferences (3 oral presentations, 3 posters).
Chapter 1: Background

Vitamin D has long been recognized for its importance in calcium metabolism and skeletal health. Over the past decade, vitamin D has been gaining increased attention for its potential role in several non-skeletal health conditions including cancer, multiple sclerosis, cardiovascular disease and diabetes. Specifically, emerging evidence suggests a potential association of low vitamin D nutritional status with increased risk of type 2 diabetes (T2DM), although the currently available data are inconsistent. Most cross-sectional and prospective studies have reported significant inverse associations of vitamin D with prevalent (1-6) and incident (7-14) type 2 diabetes, respectively. However, some studies have reported no association (15-20). In addition, only two randomized controlled trials have assessed the effects of vitamin D supplementation on incident diabetes, with negative results reported (21,22). Yet it is important to note that both these studies were post-hoc analyses which used imprecise self-reported data to determine diabetes status. Therefore, evidence for the role of vitamin D in type 2 diabetes is currently inconsistent and insufficient to determine causation.

Investigations into the role of vitamin D in the etiology of type 2 diabetes have also involved the examination of its association with both insulin resistance (IR) and beta (β)-cell dysfunction, which are the primary pathophysiological disorders underlying type 2 diabetes. The majority of studies to date have been cross-sectional in nature, and although most studies found a significant association of lower vitamin D nutritional status with greater insulin resistance and poorer β-cell function (2,23-30), some studies have also reported no association (24,31-37). In addition, previous studies often used suboptimal measures of insulin resistance and β-cell function, and included predominantly Caucasian populations. Therefore, the objective for the first study of this thesis was to examine the cross-sectional association of serum 25-hydroxyvitamin D [25(OH)D], which is the best measure of vitamin D nutritional status, with validated measures of insulin resistance and β-cell function in a multi-ethnic population at risk of type 2 diabetes (Chapter 5).

In addition to a potential role for vitamin D in insulin resistance and β-cell function, there is also emerging evidence that low 25(OH)D levels may be associated with increased risk of the metabolic syndrome (MetS), which represents a cluster of risk factors for type 2 diabetes.
Yet, the literature in this area is largely cross-sectional in nature, with most studies reporting a significant inverse association of 25(OH)D with metabolic syndrome prevalence (38-50). However, some studies have also reported no association (51-57). In addition, studies have varied in the use of different metabolic syndrome definitions and often only the traditional components of the metabolic syndrome definition were assessed in relation to 25(OH)D. Given increasing attention regarding the importance of non-traditional components (kidney dysfunction, non-alcoholic fatty liver disease (NAFLD) and inflammation) in the metabolic syndrome and in the etiology of type 2 diabetes, the potential role for 25(OH)D in these conditions is of interest. Currently however, data on the association of 25(OH)D with these non-traditional metabolic syndrome components are limited and inconsistent. Therefore, given these limitations in the literature, the objective for the second study in this thesis was to examine the cross-sectional association of 25(OH)D with the metabolic syndrome and its traditional and non-traditional components (Chapter 6).

In addition to the cross-sectional literature regarding vitamin D and diabetes related traits, there are very limited data from prospective studies, with only two studies assessing the association of baseline 25(OH)D with insulin resistance after 5-10 years of follow-up (11,58). Further, no study has yet examined the prospective association of 25(OH)D with β-cell function. Therefore, the third study of this thesis assessed the association of baseline 25(OH)D with insulin resistance and β-cell function after approximately three years of follow-up (Chapter 7). This study helped establish whether there is a temporal relationship of 25(OH)D with insulin resistance and β-cell function.

Finally it is notable that all observational studies to date examining the association of 25(OH)D with insulin resistance and β-cell function have included only a single measure of 25(OH)D and therefore do not allow for the assessment of changes in 25(OH)D and its effect on these disorders over time. Yet it is well understood that 25(OH)D levels vary by seasons throughout the year in those residing at northern latitudes, due to the reliance of 25(OH)D synthesis on sunlight exposure. Further, it has been postulated that such changes in 25(OH)D levels may have tissue-specific adverse effects (59). However, the effect of seasonal changes in 25(OH)D on measures of insulin resistance and β-cell function has not yet been
investigated. Therefore the last study of this thesis was designed to examine the effect of seasonal changes in 25(OH)D on insulin resistance and β-cell function over time (Chapter 8).
Chapter 2 : Literature Review

2.1 Type 2 Diabetes

2.1.1 Background

Diabetes is a serious public health problem that is reaching epidemic proportions globally (60). It is a chronic condition affecting an estimated 366 million people worldwide in 2011, and with prevalence rapidly increasing it is expected that 552 million will have the disease by 2030 (61). This dramatic increase in diabetes prevalence can be attributed to various factors including increased life expectancy, improved medical management, and an increase in the incidence of the disease. The economic impact of diabetes is extremely burdensome, estimated to have cost Canada’s healthcare system approximately $12.2 billion in 2010, with both direct and indirect costs expected to increase by another $4.7 billion by 2020 (62). Individuals with this chronic disease are at a significantly increased risk of numerous complications including heart disease, stroke, kidney disease, blindness and limb amputations, as well as premature death (63). Often these complications are the result of sustained high blood sugar levels due to uncontrolled or improperly managed diabetes.

2.1.2 Pathophysiology

Type 2 diabetes mellitus accounts for approximately 90 per cent of all diabetes cases. It is characterized by hyperglycemia, and thus its diagnosis is determined based on a fasting plasma glucose (FPG) level of ≥ 7.0 mmol/L or a 2-hour plasma glucose level (2hPG) ≥ 11.1 mmol/L in a 75 gram oral glucose tolerance test (OGTT) (64,65). Type 2 diabetes is a progressive disease, where individuals with normal glucose tolerance (NGT) progress to impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) and then to overt type 2 diabetes. Prediabetes encompasses either impaired fasting glucose, impaired glucose tolerance or both, as blood glucose levels are higher than normal but not yet high enough for a diagnosis of type 2 diabetes. It has been estimated that approximately six million Canadians are living with prediabetes, many of whom go on to develop type 2 diabetes (62).
Type 2 diabetes is a multifactorial metabolic disorder which arises from two primary underlying pathophysiological disorders: insulin resistance and pancreatic β-cell dysfunction. Insulin resistance refers to decreased insulin action at the level of the liver, adipose tissue and muscle (66). β-cell dysfunction is defined as the suboptimal secretion of insulin from the β-cells to meet the needs of the body in maintaining glucose homeostasis (67). Both insulin resistance and β-cell dysfunction have been shown to predict the development of incident type 2 diabetes independently of other risk factors for the disease (68-71). In most cases, early in the progression to type 2 diabetes, insulin resistance is established but glucose tolerance remains normal due to a compensatory response from pancreatic β-cells which will increase insulin secretion to maintain glucose homeostasis. However, over time and due to various genetic and acquired factors (discussed below), this compensatory response is impaired and the resulting β-cell dysfunction eventually leads to the elevation of blood glucose concentrations into the diabetes range (72,73).

2.1.3 Risk Factors

Both genetic and environmental factors are thought to play important roles in type 2 diabetes risk. Studies conducted in monozygotic twins and in individuals with affected first-degree relatives demonstrated the heritability of this disease, which has been estimated to be approximately 50% (74-77). In addition, recent genome-wide association studies (GWAS) have reported over 40 confirmed diabetes-associated loci (78-80). Therefore, it is clear that genetic factors play an important role in type 2 diabetes risk. However, several environmental/lifestyle factors also play a significant role in determining risk of this chronic disease. Obesity, a sedentary lifestyle, smoking, older age, and low socioeconomic status (SES) are all well-known risk factors for type 2 diabetes (81-83). Certain ethnic groups, including African American, Hispanic, South Asian and Aboriginal peoples have an increased risk of type 2 diabetes compared to Caucasians, which may be attributable to genetic or environmental factors. In addition, several dietary factors (e.g. coffee, whole grains, dairy, quality of carbohydrate and fat) have also been shown to have a protective effect on diabetes risk (84-89), while some dietary factors (e.g. sugar-sweetened beverages, processed meat) are associated with increased risk (90,91).
2.1.3.1 Metabolic Syndrome

Type 2 diabetes and cardiovascular disease (CVD) emerge from a number of cardiometabolic risk factors that are known to cluster together more than would be expected by chance (92). Although this risk factor cluster has been given a number of different names and defined variably, the term metabolic syndrome and its criteria is now widely agreed upon (93). The risk factors included in the most widely accepted definitions of the metabolic syndrome are as follows: raised blood pressure, dyslipidemia (high triglycerides and low high-density lipoprotein cholesterol (HDL-C)), elevated fasting glucose, and central obesity. Various diagnostic criteria for the metabolic syndrome have been put forward from different organizations including the World Health Organization (WHO) (94), International Diabetes Federation (IDF) (95), the American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) (96) and the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) (97). Although the first international definition from the World Health Organization (1998) was the most complex due to the required assessment of insulin resistance from the hyperinsulinemic euglycemic clamp procedure (94), the primary differences between these definitions were whether a component was required for the definition (i.e. insulin resistance or abdominal obesity), and how abdominal obesity was measured and defined (98). Recently, a harmonized definition of the metabolic syndrome was developed (99), in which the metabolic syndrome was defined as present if the subject had at least three of the following criteria: elevated waist circumference (≥ 102 cm for men and ≥ 88 cm for women if European-origin; ≥ 90 cm for men and ≥ 80 cm if non-European), elevated triglycerides (≥ 1.7 mmol/L or drug treatment), reduced high-density lipoprotein cholesterol (≤ 1.0 mmol/L for males and ≤ 1.3 mmol/L in females, or drug treatment), elevated blood pressure (systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg, or drug treatment), and elevated fasting glucose (≥ 5.6 mmol/L or drug treatment). Due to the existence and use of several metabolic syndrome definitions, it has been difficult to adequately compare the prevalence of the metabolic syndrome across populations. Looking at a recent study conducted among a representative sample of Canadians 18 years of age and older, approximately 19.1% had the metabolic syndrome, using the NCEP ATPIII criteria (100), with prevalence increasing with age. Although controversy exists regarding the clinical utility of the metabolic syndrome concept (101,102), it continues to be widely
considered as a useful construct given that it significantly predicts a five-fold and two-fold increased risk for type 2 diabetes and cardiovascular disease respectively, compared to those without the syndrome (103-106).

2.2 Insulin Resistance and Beta-cell Dysfunction

2.2.1 Definitions and Role in DM Etiology

Both insulin resistance and β-cell dysfunction represent early pathophysiological conditions in the natural history of type 2 diabetes. Insulin resistance refers to a condition where the normal action of insulin to lower circulating glucose concentrations, stimulate glucose utilization and suppress hepatic glucose production, is impaired (107). In the normal physiological response to increases in blood glucose, insulin secreted from β-cells binds to insulin receptors on the cell membrane of insulin-responsive tissues, which subsequently induces a signalling transduction cascade to allow for the transport of glucose into the cell for glucose utilization. However, in an insulin resistant state, there may be defects at the insulin receptor site or in the signalling pathway, which results in impaired insulin action and thus lower amounts of glucose being transported into the cell. Risk factors for insulin resistance resemble those of type 2 diabetes and include family history of diabetes, obesity, low physical activity, and older age (108). In addition, more recently described risk factors for insulin resistance include subclinical inflammation (109,110), and certain lifestyle factors such as smoking, stress, and low consumption of fibre and magnesium (111-114). Polycystic ovary syndrome and non-alcoholic fatty liver disease have also been reported as conditions which are characterized by insulin resistance (115,116).

Beta-cells make up 65-80% of the cells in the pancreatic islets, and their primary function is to make, store and release insulin to maintain glucose homeostasis in the body. In normal physiology, increases in insulin resistance are accompanied by increases in insulin secretion (117). This compensatory increase in insulin secretion in response to insulin resistance can be maintained as long as β-cell function is not impaired. Over time, however, with the increased demand on β-cells to increase insulin secretion and due to a variety of genetic and environmental factors (discussed below), the β-cell compensatory response is impaired in
some individuals resulting in β-cell dysfunction and insufficient insulin secretion (72,73,118). In the presence of β-cell dysfunction, the amount of insulin secreted cannot overcome the insulin resistance in multiple tissues, resulting in hyperglycemia (72). Therefore, although both insulin resistance and β-cell dysfunction contribute to the progression of type 2 diabetes, it is actually β-cell dysfunction that is crucial to the development of the disease because diabetes cannot occur without an impairment of insulin secretion (72,119). In contrast to insulin resistance, much less is known about the etiology of β-cell dysfunction but both genetic and environmental factors are thought to play a role. Some plausible risk factors which have recently been identified include glucotoxicity to the β-cell which would result from prolonged chronic hyperglycemia (120), lipotoxicity from the elevated free fatty acid levels that frequently coexist in individuals with increased adiposity and insulin resistance (120), chronic subclinical inflammation and oxidant stress (121), excess visceral adipose tissue (122,123), insulin resistance of the β-cells (120), and reduced adiponectin (124,125). Genetics and family history are also thought to play a role in determining risk of β-cell dysfunction (126,127). More specifically, GWAS conducted to search for novel diabetes susceptibility genes have identified mostly β-cell related loci (78,79,128).

Therefore, although some etiological risk factors for insulin resistance and β-cell dysfunction have been identified, gaps still remain in understanding the etiology of these disorders. Furthermore, factors related to the longitudinal progression of these disorders have received limited study.

2.2.2 Measurement of Insulin Resistance and β-cell Function

A variety of methods and tests are available for the measurement of insulin resistance and β-cell function. The decision to use a specific test can depend on the investigator’s purposes (clinical or research) and/or the feasibility of the various techniques based on available resources including funds, equipment, and the number of subjects. It is important to note that both insulin resistance and β-cell function are continuous traits and no validated cut-offs exist for clinical diagnostic purposes at the present time.
The hyperinsulinemic euglycemic clamp is considered the gold standard for measuring insulin resistance (129). During this procedure, intravenous insulin is given at a constant rate to raise and maintain insulin levels, while glucose is also intravenously infused at variable rates to ensure euglycemia. The steady-state at which glucose is infused (M) correlates with insulin resistance (130). However, while this method provides a valuable measure of insulin resistance, it is time- and labour- intensive, as well as expensive, and therefore not suitable for use in large populations. The frequently sampled intravenous glucose tolerance test (FSIVGTT) is another, slightly simpler, measure of insulin resistance and correlates well with that of the hyperinsulinemic-euglycemic clamp (131). Although the intravenous glucose tolerance test is generally thought to be less demanding than the euglycemic clamp, it can still be time-consuming (~3-4 hours) and is therefore not ideal in studies with large sample sizes. A variety of alternative indices have been developed to simplify the measurement of insulin resistance by using plasma glucose and insulin concentrations under fasting conditions and during an oral glucose tolerance test. A widely used measure of insulin resistance is the homeostasis model assessment of insulin resistance (HOMA-IR), which is derived from fasting plasma glucose and fasting plasma insulin concentrations (132), is an index of hepatic insulin resistance and correlates well with insulin resistance measured by the hyperinsulinemic-euglycemic clamp (133). In an oral glucose tolerance test, a subject in the fasted state consumes an oral dose of 75 grams of glucose dissolved in water. Blood glucose and insulin concentrations are then measured after two hours, and such measurements can also be made at more frequent intervals within the two hours for different research purposes. Indices of whole body insulin sensitivity can be obtained from these oral glucose tolerance test data, including the insulin sensitivity index (ISI or ISOGTT) (134), which correlates well with insulin sensitivity measured using the hyperinsulinemic euglycemic insulin clamp (135). In fact, ISOGTT has been shown to be more strongly correlated with insulin sensitivity from using the euglycemic clamp compared to HOMA-IR (136,137), and this index has shown the strongest predictive ability of type 2 diabetes risk compared to several other simple insulin resistance indices in the fasted state or during an oral glucose tolerance test (138).

The gold standard for measuring β-cell function, or more specifically insulin secretion, is the hyperglycemic clamp, where the insulin response to a constant level of hyperglycemia is evaluated (129). In addition, numerous studies have shown that loss of first-phase insulin
secretion is a strong predictor of type 2 diabetes (139-141). However, similar to the hyperinsulinemic-euglycemic clamp, this procedure is laborious, time-consuming and expensive and therefore not ideal for use in large populations. The frequently sampled intravenous glucose tolerance test has also been used to measure insulin secretion by examining the acute (0-10 min) insulin response (AIR), which has been shown to correlate with the first-phase insulin response during the hyperglycemic clamp (142). The disposition index (DI) is another measure of β-cell function which was developed to capture the hyperbolic relationship between insulin secretion and insulin sensitivity (143), and is defined by the product of the acute insulin response to glucose (AIRg) and the insulin sensitivity index (Si) obtained during a frequently sampled intravenous glucose tolerance test (117). But, as mentioned previously, the intravenous glucose tolerance test is not ideal for large epidemiological studies. Therefore, indices of insulin secretion during an oral glucose tolerance test have been developed, which better reflect the physiological route of glucose administration and which can also be used in large study populations. A widely used measure of first-phase insulin secretion is the insulinogenic index (IGI; (30 min insulin - fasting insulin) / (30 min glucose - fasting glucose)) (144), which correlates well with gold standard measures of insulin secretion (137,144,145) and has been shown to predict the development of type 2 DM (146). Measures of insulin secretion must take into account the degree of insulin sensitivity, and so an oral glucose tolerance test-based measure of β-cell function used in large studies including the Diabetes Prevention Program (DPP) (147) and the GENNID (Genetics of Non-Insulin Dependent Diabetes Mellitus) study (148) is the insulinogenic index divided by HOMA-IR. In addition, given that estimation of the disposition index requires a frequently sampled intravenous glucose tolerance test, recently an oral glucose tolerance test-based measure of β-cell function analogous to the disposition index was developed, termed the Insulin Secretion Sensitivity Index-2 (ISSI-2) (149). ISSI-2 has been shown to be strongly correlated with intravenous glucose tolerance test-derived disposition index (150).

Clearly there is a wide variety of techniques available for assessing insulin resistance and β-cell function. Each method has its own set of advantages and limitations and the choice of method is based on research needs, available resources, and feasibility.
2.3 Vitamin D

2.3.1 Background

Vitamin D is the term used to represent a group of fat-soluble compounds, in which the two major forms are vitamin D$_2$ (or ergocalciferol) and vitamin D$_3$ (or cholecalciferol). It is a 27-carbon seco-steroid whose molecular structure resembles that of classic steroid hormones including estradiol, cortisol and aldosterone (151). Vitamin D has long been recognized for its beneficial role in calcium regulation and metabolism. In children, vitamin D deficiency causes rickets, which is a bone disorder characterized by poor mineralization of the skeletal matrix resulting in growth retardation and skeletal deformities including bony projections along the rib cage (rachitic rosary) and bowed legs or knocked knees (152,153). In adults, vitamin D deficiency leads to osteomalacia which is a mineralization defect causing aching bone pain and muscle weakness (153). The discovery of the beneficial effects of vitamin D on bone health traces back to the end of the 19th century, when over 90% of children who lived in the industrialized cities of both North America and Europe had rickets, which led to the discovery of potential treatments, including cod liver oil and sunlight (154), both of which are excellent sources of vitamin D. The introduction of UV radiation of foods for the treatment and prevention of rickets made vitamin D the new miracle vitamin in the 1930s, and led to vitamin D fortification of milk and many other food products (154). However in the early 1950s, reports of hypercalcemia in infants and young children were thought to be due to vitamin D intoxication from milk (155,156). As a result, vitamin D fortification was banned in most European countries at this time. Currently, there remains limited food fortification of vitamin D in Europe, however, many countries including Canada and the United States have some mandatory fortification (e.g. milk, margarine) and allow optional fortification of other foods (157).

Emerging evidence over the past decade has suggested a potential role for vitamin D in several non-skeletal health conditions and disease states including cancer, autoimmune disorders, as well as cardiovascular disease and type 2 diabetes. However a variety of issues limit the interpretation and application of the current literature, including uncertainty
regarding cutpoints to define optimal or adequate vitamin D levels and methods for the accurate measurement of vitamin D. A discussion of these issues can be found below.

2.3.2 Sources of Vitamin D

The primary source of vitamin D for most people is the cutaneous synthesis of vitamin D through sunlight exposure, particularly solar ultraviolet B (UVB) radiation (between 290-315 nm wavelengths) (158). In addition to this endogenous production of vitamin D, humans can also obtain vitamin D from the food supply. Dietary sources of vitamin D can originate from UV irradiation of the plant sterol, ergosterol, which is found in the cell membranes of yeast and fungus, and results in vitamin D2 (or ergocalciferol), or from animal sources (vitamin D3 or cholecalciferol). Major sources of naturally containing vitamin D (vitamin D2 or D3) include cod liver oil (which provides between 400 IU of vitamin D3 per teaspoon), oily fish including wild salmon (600–1000 IU of vitamin D3 per 3.5 oz serving) and mackerel (250 IU of vitamin D3 per 3.5 oz serving), and sun-dried shitake mushrooms (1600 IU of vitamin D2 per 3.5 oz serving) (153). However, given the limited amount of vitamin D naturally present in the food supply, many countries including Canada fortify various foods with vitamin D (157,159). In Canada specifically, foods with mandatory vitamin D fortification include milk (100 IU per 250 mL) and margarine (530 IU per 100 g). Some orange juice products, plant milk products, and infant formulas can also be fortified with vitamin D (159). Vitamin D supplements, whether as a pill or as a liquid solution, also represent a predominant non-UV source through which vitamin D can be obtained, and such supplements (vitamin D2 or vitamin D3) are readily available in Canada. Although there has been discussion regarding the effectiveness of vitamin D2 versus vitamin D3, most studies have demonstrated that vitamin D3 is less effective than vitamin D3 (160-165). However, Holick et al. (2008) reported that vitamin D2 and vitamin D3 supplementation were equally effective in maintaining serum 25(OH)D levels (166). Yet a recent meta-analysis of seven randomized controlled trials (RCT) found that vitamin D3 is more effective than vitamin D2 at raising serum 25(OH)D concentrations (167), and it is generally recommended that cholecalciferol (vitamin D3) be used for nutritional and clinical purposes (163).
2.3.3 Metabolism of Vitamin D

Solar ultraviolet exposure results in the cutaneous synthesis of cholecalciferol (vitamin D₃) from its precursor 7-dehydrocholesterol (7-DHC) in the skin. Specifically, sunlight exposure results in the synthesis of previtamin D₃, which then undergoes nonenzymatic isomerization to form vitamin D₃. Vitamin D, whether endogenously synthesized or ingested, must undergo two hydroxylations in the body before it becomes an active hormone. Once in the body, vitamin D is transported in circulation by binding to the vitamin D binding protein (DBP), and first travels to the liver where it is hydroxylated by the cytochrome P450 enzyme vitamin D 25-hydroxylase (CYP2R1 and/or CYP27A1) to 25-hydroxyvitamin D (25(OH)D, calcidiol) which is the major circulating form of vitamin D in the body (168,169). Following this, 25(OH)D then travels to the kidney where it is further hydroxylated by the enzyme 25-hydroxyvitamin D 1-alpha-hydroxylase (25(OH)D-1αOHase or CYP27B1) to form the active vitamin D metabolite, 1,25-dihydroxyvitamin D (1,25(OH)₂D, calcitriol) (170). See Figure 2-1 for a diagram depicting vitamin D synthesis and metabolism. Circulating 25(OH)D can be taken up by tissues through two different mechanisms; free circulating 25(OH)D can diffuse directly across cell membranes, or 25(OH)D bound to DBP in circulation can enter certain tissues, particularly the kidney, by the endocytic receptor megalin (171). The hydroxylation of vitamin D to 25(OH)D in the liver is not tightly regulated, whereas the renal production of 1,25(OH)₂D is tightly regulated by calcium, parathyroid hormone (PTH), phosphorus and 1,25(OH)₂D itself (172). When blood calcium levels are insufficient, PTH levels increase which signal an increase in calcitriol synthesis that subsequently increases intestinal absorption of calcium (173). In addition, when phosphate levels are high, this stimulates the production of fibroblast growth factor-23 (FGF-23) in the bone, which then inhibits calcitriol synthesis in the kidney (174). Further, when 1,25(OH)₂D levels are sufficient, this metabolite induces the catabolic enzyme 1,25-dihydroxyvitamin D-24-hydroxylase (CYP24A1) to form 24,25-dihydroxyvitamin D (24,25(OH)₂D) in the kidney, where it is further metabolized to biologically inactive, water-soluble calcitroic acid and other carboxylated products that are excreted by the kidney (168,175). In addition to the renal activation of vitamin D, the 25(OH)D-1αOHase (CYP27B1) enzyme is also present in numerous non-renal tissues and cells including skin, brain, prostate, pancreas and macrophages (176), which therefore results in the extra-renal synthesis of the active
**Figure 2-1.** Synthesis and metabolism of vitamin D, from photoproduction or dietary intake of vitamin D$_2$/D$_3$.

1,25(OH)$_2$D metabolite. Regulation of non-renal calcitriol synthesis is largely unknown, although CYP27B1 mRNA is highest in the kidney (177).

The biological activity of 1,25(OH)$_2$D can be classified as either genomic or non-genomic. It has been estimated that up to 3% of the human genome is directly or indirectly controlled by the vitamin D system (178). In addition, it has been estimated that locally synthesized 1,25(OH)$_2$D can control up to 2000 genes that are involved in a variety of processes including cell growth, immunity, cell proliferation and inflammation (179,180). The genomic actions of 1,25(OH)$_2$D require the binding of calcitriol to a high-affinity receptor, the vitamin D receptor (VDR) which is a member of the nuclear hormone receptor superfamily and acts as a ligand-activated transcription factor (177). Although the vitamin D receptor can be found in organs involved in calcium metabolism and homeostasis including the intestine, bone, kidney and parathyroid glands, it has also been identified in numerous other tissues and cells (i.e. heart, breast, colon, prostate, and pancreas) (181,182). When 1,25(OH)$_2$D binds to the vitamin D receptor, this forms a heterodimer complex with the 9-cis retinoic acid nuclear retinoid-X-receptor (RXR). The vitamin D receptor/retinoid X receptor complex then binds to the vitamin D response element (VDRE) in the promoter region of target genes. A coactivator complex of proteins is then recruited and binds with the heterodimeric vitamin D receptor/retinoid X receptor complex which corresponds with the RNA polymerase for gene transcription (177). In vitamin D responsive genes, the unliganded vitamin D receptor/retinoid X receptor complex will still bind to the vitamin D response element of the promoter region of the specific target gene, but a co-repressor complex is then recruited which will repress gene transcription (181). In addition to its genomic actions, calcitriol also mediates rapid non-genomic effects, which occur via the binding of 1,25(OH)$_2$D to a cytoplasmic membrane vitamin D receptor. These non-genomic actions of calcitriol play an important role in nuclear transcription activity, as well as membrane-associated events, such as increasing calcium uptake, releasing intracellular calcium stores, as well as stimulating protein kinase C activity (180,183).
2.3.4 Factors Influencing 25(OH)D levels

Numerous factors can influence the synthesis of vitamin D and its concentrations in the body. The solar zenith angle (SZA), which is a function of time of day, time of year (season), and latitude, is an important factor influencing the association of sun exposure and vitamin D (184). The distance through which UV radiation must travel through the atmosphere is longer early in the morning and late in the afternoon, which results in diminished UV radiation reaching the earth’s surface during these times, compared to that at noon time. The SZA also varies throughout the year, as it is smallest in the summer when the sun is directly overhead, resulting in higher UVB radiation reaching the earth’s surface compared to the winter when the SZA is the largest, due to the lower angle of the sun. Latitude is also an important factor given that the SZA is smaller near the equator and increases as one moves toward the poles. Therefore, there is little cutaneous vitamin D production early in the morning and later in the afternoon, or during the winter months in those living above 35° north latitude in the northern hemisphere (185,186).

Skin pigmentation is another important factor determining cutaneous vitamin D synthesis, as the absorption of UVB radiation is decreased with greater melanin content. Individuals with darker skin have a higher melanin content which absorbs UV photons and hence competes with 7-dehydrocholesterol (187). Avoidance of sun exposure, covering most of one’s body with clothing, and sunscreen use also reduce cutaneous vitamin D synthesis (188,189).

Body composition is also a significant determinant of vitamin D levels, as numerous studies have consistently shown that those with increased adiposity have lower vitamin D levels (190-192), which is thought to be due to the sequestration of vitamin D in adipose tissue, given that it is a fat-soluble vitamin (192,193). A recent study, however, suggests that volumetric dilution as a function of body weight explains low 25(OH)D levels in those with a larger body size (194), which is supported by data indicating that vitamin D is stored in both adipose tissue and muscle (195,196). In addition, those with malabsorption disorders resulting in reduced fat absorption (i.e. cystic fibrosis, celiac disease, Chron’s disease) have decreased vitamin D bioavailability due to an impaired ability to absorb vitamin D (197). Those with liver or kidney disease also have reduced 25(OH)D or 1,25(OH)₂D levels.
respectively due to impairments in vitamin D metabolism (198,199). Certain medications including anticonvulsants, glucocorticoids and anti-rejection medications can also influence vitamin D levels, as use of these medications increases the catabolism of the 25(OH)D and 1,25(OH)₂D metabolites (186,200,201). In addition, individuals who have poor vitamin D intake from their diet have reduced vitamin D levels, particularly in areas with seasonally fluctuating UVB radiation (185,202,203).

Genetic factors are also a significant determinant of 25(OH)D levels and contribute to the inter-individual variation in 25(OH)D, with heritability thought to significantly influence both concentrations and variability (204-206). In addition, two recent genome-wide association studies reported a significant association of circulating 25(OH)D concentrations with polymorphisms for genes encoding the vitamin D binding protein and enzymes involved in the metabolic pathway of vitamin D (207,208). These findings were confirmed in a recent systematic review (209), which also reported a significant association of polymorphisms in the vitamin D receptor gene and 25(OH)D concentrations. Although current data are limited and inconsistent regarding the effect of genetic polymorphisms in a wide variety of genes involved in vitamin D synthesis, metabolism, and action, research activity in this area is increasing.

2.3.5 Assessing Vitamin D Nutritional Status

Serum or plasma 25(OH)D (both 25(OH)D₂ and 25(OH)D₃) is considered the best measure of vitamin D nutritional status, as it is the major circulating form of vitamin D in the body and is reflective of both dietary intake and cutaneous synthesis. The half-life of serum 25(OH)D (approximately 1-2 months (163)) is longer than that of 1,25(OH)₂D (approximately 10-24 hours (210)), as a result of its stability in circulation when bound to DBP. In addition, renal production of 1,25(OH)₂D is tightly regulated (211), and 1,25(OH)₂D levels are often normal or even slightly elevated in the presence of 25(OH)D insufficiency. There has been some controversy over whether the measurement of 25(OH)D is adequate for making dietary recommendations given disagreement regarding the optimal serum level for this metabolite (discussed below), and also given significant variability in the assays measuring serum 25(OH)D (212,213). Although other potential functional markers of
vitamin D status have been suggested including PTH, bone mineral density and calcium absorption (214,215), a recent systematic review of these existing markers confirmed that circulating 25(OH)D was a robust and reliable marker of vitamin D status (216).

2.3.6 Measurement of Serum 25(OH)D

The measurement of 25(OH)D levels in the body can be challenging due to the lipophilic nature of this metabolite, its strong binding affinity to protein, and also given its two structurally similar forms, 25(OH)D$_2$ and 25(OH)D$_3$ (217,218). Further, there is little agreement on which assay should be used to measure circulating 25(OH)D. Several assays are available for the measurement of 25(OH)D, including manual methods such as competitive protein-binding assays, radioimmunoassays (RIA), high performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS/MS), as well as automated competitive chemiluminescence immunoassays. Although HPLC and LC-MS/MS are often considered the gold standard, these procedures are time- and labour-intensive, costly, and require an internal standard, large sample volumes, as well as a well-trained technologist to perform these procedures accurately (218). Therefore, the RIA has been the most widely used method for measurement of 25(OH)D, and the DiaSorin RIA was the first test approved for clinical diagnosis by the US Food and Drug Administration (FDA) (218). However, the RIA is limited in that it is a manual method and also involves the use of radioactive materials. More recently, automated competitive chemiluminescence immunoassay procedures have been developed which can provide higher-throughput capacity and require lower sample volumes. The use of such automated immunoassays have been increasing in popularity, and recently the FDA approved DiaSorin’s Liaison 25(OH)D Total chemiluminescent immunoassay for clinical use. In addition, this method has shown strong correlation with the DiaSorin RIA reference method (219).

Given the existence of several assays for the measurement of 25(OH)D, the International External Quality Assessment Scheme for Vitamin D metabolites (DEQAS, Northwest Thames, United Kingdom) was established in 1989 to monitor the reliability of 25(OH)D assays (220). DEQAS as well as numerous other studies have reported on the large inter-assay and inter-laboratory variability in the measurement of 25(OH)D (212,213,221,222),
which has resulted in the call for a better reference material and the need for the standardization of 25(OH)D methods (222-224). Although there has been much development in the area of 25(OH)D assays and continued monitoring of their reliability, there remains a lack of consensus regarding the best and most accurate method, and therefore, further research in this area is needed.

2.3.7 Recommended 25(OH)D Levels

Another critical issue in the vitamin D literature is the lack of agreement regarding what constitutes an optimal 25(OH)D level. This lack of consensus has been confounded by the substantial inter-assay variability of 25(OH)D assays as well as the fact that various tissues and disease states likely have different optimal 25(OH)D cut-offs. For example, it is generally well accepted that a serum 25(OH)D level less than 25 nmol/L impairs normal bone mineralization and can cause rickets or osteomalacia. Higher cut-offs have been proposed, including 25(OH)D levels of 75-80 nmol/L which relate to maximum calcium absorption (225,226), as well as for other outcomes including cancer (227). Cut-off levels of 25(OH)D to indicate adequate or optimal vitamin D status are needed for clinical use and for making public health recommendations. In November 2010, the Institute of Medicine (IOM) released a much anticipated report on new vitamin D recommendations, replacing the outdated 1997 Dietary Reference Intakes (DRIs) for calcium and vitamin D (228). After reviewing the evidence, the Institute of Medicine committee concluded that bone health was the only outcome that was causally implicated by vitamin D, and therefore updated the dietary reference intakes based on this clinical outcome alone. They concluded that there was insufficient compelling evidence that vitamin D played a significant beneficial role on extraskeletal outcomes, due to the lack of data from high quality randomized controlled trials. The new dietary reference intakes are as follows: serum 25(OH)D level of 40 nmol/L is the median population requirement (estimated average requirement, EAR) and 25(OH)D of at least 50 nmol/L meets the needs of 97.5% of the population (recommended dietary allowance, RDA). The Institute of Medicine committee then set intakes of vitamin D which would be required to achieve these concentrations of 25(OH)D assuming minimal sun exposure (see Table 2-1). Despite these recommendations for adequate 25(OH)D concentrations and optimal vitamin D intake for skeletal health, many experts have contested
the report’s conclusions and state that 25(OH)D levels of at least 75 nmol/L are required for overall beneficial health outcomes (229-232). Therefore, despite the overdue updated dietary reference intakes for vitamin D, controversy still remains regarding what constitutes an adequate 25(OH)D concentration for optimal health.

Table 2-1. 2011 Dietary Reference Intakes for vitamin D by life stage

<table>
<thead>
<tr>
<th>Life stage group</th>
<th>Vitamin D</th>
<th>Serum 25(OH)D level (nmol/L) (corresponding to the RDA)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>UL (IU/d)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3 yr</td>
<td>600</td>
<td>50</td>
<td>2500</td>
</tr>
<tr>
<td>4-8 yr</td>
<td>600</td>
<td>50</td>
<td>3000</td>
</tr>
<tr>
<td>9-13 yr</td>
<td>600</td>
<td>50</td>
<td>4000</td>
</tr>
<tr>
<td>14-18 yr</td>
<td>600</td>
<td>50</td>
<td>4000</td>
</tr>
<tr>
<td>19-30 yr</td>
<td>600</td>
<td>50</td>
<td>4000</td>
</tr>
<tr>
<td>31-50 yr</td>
<td>600</td>
<td>50</td>
<td>4000</td>
</tr>
<tr>
<td>51-70 yr</td>
<td>600</td>
<td>50</td>
<td>4000</td>
</tr>
<tr>
<td>71+ yr</td>
<td>800</td>
<td>50</td>
<td>4000</td>
</tr>
<tr>
<td>Pregnant or lactating</td>
<td>600</td>
<td>50</td>
<td>4000</td>
</tr>
<tr>
<td>14-18 yr</td>
<td>600</td>
<td>50</td>
<td>4000</td>
</tr>
<tr>
<td>19-50 yr</td>
<td>600</td>
<td>50</td>
<td>4000</td>
</tr>
<tr>
<td>Infants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-6 months</td>
<td>400&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50</td>
<td>1000</td>
</tr>
<tr>
<td>6-12 months</td>
<td>400&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50</td>
<td>1500</td>
</tr>
</tbody>
</table>

EAR for vitamin D was 400 IU/d for all life-stage groups.  
<sup>a</sup> Measures of serum 25(OH)D levels corresponding to the RDA and covering the requirements of at least 97.5% of the population  
<sup>b</sup> UL (Tolerable Upper Intake Level) indicates the level above which there is risk of adverse events. The UL is not intended as a target intake  
<sup>c</sup> Reflects AI (Adequate Intake) reference value rather than RDA. RDAs have not been established for infants.

*Modified from Institute of Medicine 2011 Report*
2.4 Vitamin D Nutritional Status and Type 2 Diabetes

As mentioned previously, in addition to the well-recognized effects of vitamin D on skeletal health, emerging evidence suggests a potential role for vitamin D in numerous other disease states and health conditions including cancer, autoimmune disorders, cardiovascular disease and type 2 diabetes. Looking specifically at type 2 diabetes, early animal studies demonstrated that calcium and magnesium, both of which are tightly regulated by the vitamin D system, were essential for insulin secretion (233-236). Similarly, animal studies also found that vitamin D deficiency was associated with impaired insulin secretion, and that vitamin D supplementation restored normal insulin secretion (237-241). In addition to this experimental data, seasonal variation in glucose and insulin concentrations (242,243), as well as seasonal variation in the diagnosis and control of type 2 diabetes has been reported, such that there is increased diagnosis and poorer glycemic control in the winter compared to that in the summer (244). This seasonal variability in type 2 diabetes related traits may be attributable to vitamin D, given the well characterized seasonal variation in serum 25(OH)D levels.

Consistent with these findings which suggest a potential role for vitamin D in type 2 diabetes etiology, numerous cross-sectional human studies have reported a significant inverse association between serum 25(OH)D and the presence of type 2 diabetes (1,2). In addition, most case-control studies have also found that patients with type 2 diabetes or impaired glucose tolerance are significantly more likely to have a lower serum 25(OH)D concentration compared to those without diabetes (3-6). However, findings have not been entirely consistent, with some studies reporting no association (15,16). In addition, a growing number of prospective studies have been conducted with most reporting a significant inverse association of baseline serum 25(OH)D with incident diabetes (7-14,245,246). This notion was supported in two recent meta-analyses of 8 (247) and 11 (248) prospective studies, which found a significant inverse association of baseline 25(OH)D with incident type 2 diabetes. However, some studies have also reported no association (17-20). These inconsistencies may be due to the use of self-reported vitamin D intake in some studies (7,19), a predicted 25(OH)D score in one study (10), and the use of self-reported diabetes status to ascertain the primary outcome (7,8,19,20). In addition, almost all prospective studies to date have assessed vitamin D status only once at baseline. In fact, only one study using
repeat measurements of 25(OH)D has been conducted (13), which found a significant inverse association of 25(OH)D with incident diabetes after 2.7 years of follow-up.

Only two RCTs investigating the effects of vitamin D (D$_2$ or D$_3$) supplementation on incident type 2 diabetes have been conducted (21,22), as the majority of trials in this area have examined measures of glycemia, insulin resistance and/or insulin secretion as the primary study outcomes (discussed below). Both of the trials conducted to date found no statistically significant effect of vitamin D$_3$ supplementation [400 (21) or 800 IU (22) daily] on incident diabetes after two to five (22) or seven (21) years of follow-up. However, these studies ascertained diabetes status using self-reported data and both were post-hoc analyses of trials that were designed to assess other conditions/diseases as the primary outcome. Clearly, additional RCTs specifically designed to assess the effect of vitamin D supplementation on risk of type 2 diabetes are needed.

2.5 Vitamin D and the Metabolic Syndrome

Given emerging evidence suggesting a potential role for vitamin D in type 2 diabetes, there has been increased interest in investigating the association of vitamin D with several diabetes risk factors, particularly the metabolic syndrome. The majority of previous studies examining an association of 25(OH)D with the metabolic syndrome have been cross-sectional in nature, with most reporting a significant inverse association of 25(OH)D with metabolic syndrome prevalence (38-50). However, some studies have also reported no association (51-57). The lack of an association reported in these studies may be due to the fact that some of these studies examined either those with established type 2 diabetes (56), study populations which were recruited based on the presence of obesity (which may have constrained the variability in the bioavailability of 25(OH)D) (52,53), or populations with year-round sunny climate which may reduce variability in 25(OH)D (51,55). One of the studies reporting no association did not measure serum 25(OH)D levels, but relied solely on dietary and supplemental sources of vitamin D (54). In addition, differences in the cutoffs used to define vitamin D insufficiency or deficiency may explain inconsistent findings (57). Some studies have also reported a significant association of parathyroid hormone (PTH) with the metabolic syndrome, independent of 25(OH)D (39,51,52,249), but other studies have also reported no
association (38,41,43,51,53). In addition to examining the role of 25(OH)D on metabolic syndrome prevalence, several studies have also included an investigation into potential associations of 25(OH)D with the traditional components of the metabolic syndrome (i.e. dyslipidemia, hypertension, etc.). However, inconsistent findings have been reported (42,44,46,47,49,250-252). Given emerging evidence of the importance of non-traditional metabolic syndrome components (kidney dysfunction, non-alcoholic fatty liver disease and inflammation) in the etiology of type 2 diabetes, the potential role for 25(OH)D in these disease conditions is of interest. Currently, however, studies assessing the role of 25(OH)D with the non-traditional components of the metabolic syndrome are limited and inconsistent (47,56,250,253-256), but research in this area has been increasing in recent years.

There is currently very limited data on the prospective association of 25(OH)D on metabolic syndrome risk over time. Forouhi et al. (2008) found that higher baseline 25(OH)D levels were significantly associated with lower metabolic syndrome risk z-scores at the 10-year follow-up after adjustment for age, sex, smoking, season, BMI and baseline metabolic syndrome z-score (p=0.048) (58). But this association was attenuated to non-significance with further multivariate adjustment (i.e. PTH, calcium, physical activity, insulin-like growth factor-1, occupational social class). A more recent study in a larger study population (n=6537) reported that for each 25 nmol/L decrease in 25(OH)D, the risk of metabolic syndrome at the 5-year follow-up increased by 23% after multivariate adjustment. However, this association between 25(OH)D and metabolic syndrome risk was attenuated to non-significance after further adjustment for HOMA-IR (257). This study also reported significant inverse associations of baseline 25(OH)D with several metabolic syndrome components at follow-up including waist circumference, triglycerides, fasting glucose and HOMA-IR.

To date, only one study has investigated the effects of increasing serum 25(OH)D on metabolic syndrome risk (258). In this study, 59 nondiabetic overweight/obese Saudi Arabian adult subjects were advised to increase their sun exposure and intake of vitamin D rich foods for one year. Overall, this study reported that an increase in 25(OH)D after one year of follow-up was associated with a parallel decrease in metabolic syndrome prevalence. However, there was no control group and serum 25(OH)D increased from only 20 to 27.5
nmol/L. Clearly, additional trials are needed to assess the effect of vitamin D supplementation on metabolic syndrome. Further, although there have been some trials investigating the effects of vitamin D supplementation on various metabolic syndrome components (259-263), results have not been consistent and therefore, additional research in this area is warranted.

2.6 Role of Vitamin D in Insulin Resistance & Beta-cell Function

2.6.1 Studies to Date

In addition to literature examining the association of vitamin D with type 2 diabetes and its risk factors, a number of studies have also investigated the role of vitamin D in the primary pathophysiological disorders underlying type 2 diabetes, specifically insulin resistance and β-cell dysfunction.

The majority of early evidence of a potential role of vitamin D with insulin resistance and β-cell function came from cross-sectional studies. Most (2,23-27), but not all (31-34,36,37), of these studies reported a significant inverse association of serum 25(OH)D with insulin resistance. Inconsistent findings have also been reported in the cross-sectional studies investigating the association of 25(OH)D with β-cell function, with some studies reporting a positive association (28-30) and some finding no significant association (2,24,31,32,35,37). However, most of the studies cited used indirect (proxy) measures of insulin resistance and β-cell function, such as fasting or post-challenge glucose or insulin, HOMA-IR, HOMA-β, or other fasting-based measures (2,23,27-30,32,33). Inadequate covariate adjustment, such as not assessing physical activity as a potential confounder, in some studies may also limit findings (264). In addition, temporality or causation cannot be inferred from such studies due to the nature of the cross-sectional study design.

In contrast to the existing cross-sectional literature examining the association of 25(OH)D with insulin resistance and β-cell function, prospective data are much more limited. Only two studies to date have investigated the association of baseline 25(OH)D with follow-up insulin resistance (11,58), with both studies reporting a significant inverse association of baseline
25(OH)D with insulin resistance after five- (11) and ten- (58) years of follow-up. No prospective study has yet examined the association of 25(OH)D with β-cell function.

Numerous intervention trials examining the effects of vitamin D (D₂ or D₃) supplementation on insulin resistance and β-cell function have been conducted but inconsistent findings have been reported (21,29,260,265-283). This may be due to differences in vitamin D dose, short study duration, as well as differences in the measures used to assess the primary study outcomes (e.g. fasting plasma glucose, HOMA-IR, HbA1c).

Only a few studies have examined the effects of vitamin D supplementation on insulin resistance using the gold standard measures, specifically the euglycemic clamp or intravenous glucose tolerance test; most of which have not demonstrated beneficial effects of vitamin D (278-282). However, it is important to note that in three of the studies, participants were supplemented with the active vitamin D metabolite, 1,25(OH)₂D or its analog (278-280). Given the local production of 1,25(OH)₂D in extra-renal tissues, it is thought that supplementation with ergocalciferol (vitamin D₂) or cholecalciferol (vitamin D₃) is favourable over calcitriol as they are substrates for the 25(OH)D-1-α-hydroxylase enzyme and can allow for local calcitriol synthesis (284,285). However, Grimnes et al. (2011) also found no effect of supplementation (20,000 IU vitamin D₃ twice weekly for 6 months) on insulin sensitivity measured using a 3-hour hyperglycemic clamp in 52 healthy subjects (281). Although Nazarian et al. (2011) did report increased intravenous glucose tolerance test-measured insulin sensitivity with high dose vitamin D₃ supplementation (10,000 IU per day), this study included only eight subjects and it was not a placebo-controlled trial (282). Similarly, inconsistent effects of vitamin D supplementation have been reported in studies using indirect indices of insulin resistance including fasting glucose/insulin or HOMA-IR (21,260,265-276,281). The only study (n=71) using an oral glucose tolerance test-derived measure of peripheral insulin sensitivity reported significant improvements with supplementation of three doses of 120,000 IU of vitamin D₃ fortnightly versus placebo after six weeks of follow-up (268). However, this study found no significant improvements in fasting-based indices of insulin resistance, highlighting the importance of examining both hepatic and peripheral insulin resistance.
Inconsistent effects of vitamin D supplementation on measures on β-cell function have also been reported (29,35,268,271,274,277,281), in that some studies reported a significant beneficial effect and some reported no effect. However, most studies have been of short duration with differing vitamin D doses, small sample sizes, and reliance on surrogate measures of β-cell function including HOMA-β and fasting/post-challenge C-peptide. These methods of assessing β-cell function are limited as HOMA-β relies on simple fasting measurements and both HOMA-β and C-peptide do not account for ambient insulin resistance, which is important given the hyperbolic (compensatory) relationship between insulin secretion and insulin sensitivity. Five of these studies utilized gold standard measures of β-cell function (274,277-279,281), but inconsistent findings were also reported. However, two of these studies (278,279) supplemented with calcitriol or its analog which, as previously mentioned, may not adequately allow for physiological effects to be observed. Although Borissova et al. (2003) reported increased first-phase insulin secretion with supplementation of 1332 IU vitamin D₃ per day, this was not a randomized placebo controlled trial, and was conducted in only 10 participants (274). More recently, however, a randomized controlled trial conducted in 92 adults found that supplementation of 2000 IU vitamin D₃ per day for 16 weeks increased β-cell function measured by the disposition index (277). Yet in another study, Grimnes et al. (2011) found no significant effect of high dose vitamin D supplementation (20,000 IU vitamin D₃ twice weekly for 6 months) on measures of insulin secretion from the hyperglycemic clamp among their participants (n=52) (281).

Only three small randomized controlled trials assessing the effect of vitamin D supplementation on insulin resistance and β-cell function have been conducted in those with established type 2 diabetes (267,270,272). Supplementation with vitamin D₃ (40,000 IU/week for 26 weeks (270), 100,000 or 200,000 IU vitamin D₃ given once (272)), or a single dose of 100,000 IU vitamin D₂ (267) did not have any effect on glucose homeostasis measures including fasting glucose, insulin, and C-peptide, HbA1c, or HOMA-IR after a follow up period of 8-26 weeks. However, sufficient conclusions cannot be drawn from these trials due to the fact that all studies were underpowered (n=26 to 61), and changes in diabetes medications were not accounted for or reported. In addition, only one study was designed for glycemic outcomes, but just about half of the planned cohort was recruited (270).
Overall, the scientific literature is currently inconsistent regarding the association of 25(OH)D with insulin resistance and β-cell function in observational studies, and there is a lack of studies allowing for the evaluation of temporal associations to be observed. In addition, data from well-designed and powered clinical trials assessing the effect of vitamin D supplementation on insulin resistance and β-cell function are lacking. Therefore, it is clear that further investigation is needed to examine a potential role for vitamin D in the disorders underlying type 2 diabetes.

2.6.2 Mechanisms

A number of potential mechanisms have been proposed to explain the association of vitamin D with type 2 DM and its related disorders. Vitamin D may directly enhance insulin action for glucose transport by stimulating the expression of insulin receptors (286), as the vitamin D response element is present in the promoter region of the insulin receptor gene (287). Vitamin D may also have indirect effects on insulin resistance by regulating insulin-mediated intracellular processes via regulation of the calcium pool (288,289). Elevated intracellular calcium can inhibit insulin-target cells from sensing brisk intracellular fluxes in calcium which are required for insulin action including glucose transport (290). It is also important to note that the primary determinants of peripheral insulin sensitivity, specifically skeletal muscle and adipose tissue, express the vitamin D receptor (180,291), and like insulin sensitivity, skeletal muscle expression of the vitamin D receptor declines with age (292). Further, these tissues have also been shown to express the 25(OH)D-1-α-hydroxylase enzyme in Wistar rats (293), allowing for local calcitriol synthesis. With regard to β-cell function, vitamin D may exert direct effects through the binding of its circulating active form (1,25(OH)₂D) to the β-cell vitamin D receptor (294,295). Alternatively, activation of vitamin D could occur within the β-cell by the 25(OH)D-1-α-hydroxylase enzyme, which has been shown to be expressed in β-cells (296). In addition, given the presence of the vitamin D response element in the promoter region of the insulin gene (287), this can translate into transcriptional activation of the insulin gene by 1,25(OH)₂D (297). Vitamin D may also exert an indirect effect on β-cell function by regulating extracellular calcium and calcium flux through the β-cell (298), as insulin secretion is a calcium-dependent process (233). Based on the association between type 2 DM and systemic inflammation (299), vitamin D may also
improve insulin sensitivity and promote β-cell function by moderating the generation and effects of cytokines (264). However, there is very limited and inconsistent data from studies examining the relationship between vitamin D and systemic inflammation associated with type 2 DM (264,300).

2.6.3 Role of Genetics

Genetic differences may help partially explain inconsistencies in the literature regarding the association of vitamin D with type 2 diabetes and its underlying disorders. Most research in this area has focussed on different genotypes related to the vitamin D receptor, the vitamin D binding protein, and the 25(OH)D-1-α-hydroxylase enzyme. Polymorphisms which have been identified in the vitamin D receptor gene, specifically TaqI, BsmI, ApaI and FokI, may be associated with type 2 diabetes, insulin resistance and/or β-cell dysfunction. However, current evidence is limited and results have been inconsistent. Studies have found significant associations of specific vitamin D receptor polymorphisms with greater insulin resistance (301-305) and insulin secretion (306-308). However, most of these studies have been conducted in predominantly Caucasian populations and have used surrogate fasting based measures of insulin resistance and β-cell function. With regard to type 2 diabetes specifically, one study has reported a higher prevalence of type 2 diabetes in those with a specific genotype for the BsmI vitamin D receptor gene compared to those without this genotype (309). The remaining studies, all of which were case-control studies, reported no significant differences in genotype frequencies for various vitamin D receptor genes in diabetics versus controls (310-315). Therefore, additional investigation into the relation between vitamin D receptor polymorphisms and risk of type 2 diabetes is warranted, particularly in different ethnic populations and in studies using gold standard measures of insulin resistance and β-cell function. Genetic variants of the DBP have also been identified, with some studies reporting an association of such polymorphisms and increased risk of type 2 diabetes (316) and insulin resistance as measured by fasting glucose or insulin levels (317,318). However, there is limited data and findings have not been consistent (317-321). Another vitamin D-related gene investigated for a possible relation with type 2 diabetes has been 25(OH)D-1-α-hydroxylase (CYP1alpha), which is responsible for the conversion of 25(OH)D to calcitriol. Yet, only one study has been conducted to date (312), which reported no significant
difference in the CYP1alpha gene or its genotypes in type 2 diabetics versus controls in a Polish population. This study did, however, find a significant association with a specific genotype of the CYP1alpha gene and type 2 diabetes in the obese subgroup, but the mechanisms of this association are not clear. More recently, another study also reported no significant association of type 2 diabetes with several single nucleotide polymorphisms associated with serum 25(OH)D level (322). Although current research is limited and inconsistent, evidence to date does suggest a possible link between genes involved in vitamin D metabolism and type 2 diabetes etiology and traits.

2.6.4 Summary/Rationale

The prevalence of diabetes is dramatically increasing, both in Canada and worldwide. In addition, given the latitude and long winters in Canada, Canadians are at an increased risk of having insufficient levels of serum 25(OH)D, with over 25% having 25(OH)D levels less than 50 nmol/L (323) and 65% with 25(OH)D less than 75 nmol/L (324). Emerging evidence suggests a potential role for vitamin D in risk of type 2 diabetes and its underlying pathophysiological disorders, specifically insulin resistance and β-cell dysfunction. Although numerous epidemiological, interventional, and biological studies have suggested a possible association of vitamin D with insulin resistance and β-cell function, findings have not been consistent. Similarly, studies examining the association of vitamin D with risk factors for diabetes including the metabolic syndrome have been increasing but findings have also been inconsistent. Many of the studies conducted to date have often been limited by inadequate sample sizes, use of suboptimal surrogate measures of outcomes, and predominantly Caucasian study populations. There have also been very few studies examining the role of vitamin D in the longitudinal progression of insulin resistance and β-cell function. In addition, given the well characterized seasonal variation in 25(OH)D levels, no study has yet examined the effect of such seasonal fluctuations in 25(OH)D on insulin resistance and β-cell function longitudinally. Therefore, there are clearly gaps in the literature and a lack of conclusive evidence regarding the association between vitamin D and risk of type 2 diabetes. This thesis will attempt to address some of the knowledge gaps, specifically by conducting cross-sectional and prospective studies using validated measures of insulin resistance and beta-cell function, by assessing the role of vitamin D with novel risk factors for type 2
diabetes, and by examining the effect of seasonal fluctuations in 25(OH)D on our outcome measures using data from a cohort of well characterized Canadian subjects at high risk for type 2 diabetes.
Chapter 3: Objectives and Hypotheses

Objective 1

The first objective of this thesis was to examine the role of vitamin D in the metabolic disorders underlying type 2 diabetes, specifically insulin resistance and β-cell dysfunction, in a multi-ethnic cohort at risk of type 2 diabetes.

Objective 1a – To assess the cross-sectional association of baseline serum 25(OH)D with baseline measures of insulin resistance and β-cell function.

Hypothesis 1a – There will be a significant inverse association of baseline serum 25(OH)D with baseline measures of insulin resistance and a significant positive association of baseline 25(OH)D with baseline measures of β-cell function.

Objective 1b – To assess the prospective association of baseline serum 25(OH)D with measures of insulin resistance and β-cell function at the three-year follow up examination.

Hypothesis 1b – There will be a significant inverse association of baseline serum 25(OH)D with follow-up measures of insulin resistance and a significant positive association of baseline 25(OH)D with follow-up measures of β-cell function.

Objective 2

The second objective of this study was to investigate the association of vitamin D with diabetes-related metabolic risk factors.

Objective 2 – To assess the cross-sectional association of baseline serum 25(OH)D with baseline measures of the metabolic syndrome and its components.

Hypothesis 2 – There will be a significant inverse association of baseline 25(OH)D with the prevalence of the metabolic syndrome at baseline. In addition, low baseline 25(OH)D will be significantly associated with various components of the metabolic syndrome.
Objective 3

The third objective of this study was to examine the effect of seasonal changes in serum 25(OH)D on measures of insulin resistance and β-cell function over time.

Objective 3 – To investigate whether seasonal fluctuations in 25(OH)D are significantly associated with insulin resistance and β-cell function during the follow-up period.

Hypothesis 3 – Individuals who have a summer then a winter clinic visit, will have significant decreases in serum 25(OH)D as well as concomitant decreases in insulin sensitivity and β-cell function, independent of potential confounders. Individuals who have a winter followed by a summer clinic visit, will have significant increases in serum 25(OH)D as well as concurrent increases or relatively less of a decrease in both insulin sensitivity and β-cell function, independent of potential confounders.
CHAPTER 4:

PROSPECTIVE METABOLISM AND ISLET CELL EVALUATION (PROMISE)

COHORT STUDY: RESEARCH DESIGN AND METHODS
Participants in the Prospective Metabolism and Islet cell Evaluation (PROMISE) cohort were recruited during the screening phase for the Canadian Normoglycemia Outcomes Evaluation (CANOE; clinical trial registration no. NCT00116922), a double-blind, randomized controlled trial of 207 individuals with impaired glucose tolerance (IGT) randomized to either rosiglitazone and metformin or placebo to determine whether this approach can prevent the development of type 2 diabetes in these high-risk subjects (325). Participants were recruited from Toronto (43° 40' N) and London (43° 02' N), Ontario, Canada, between May 2004 and December 2006. Each center promoted the study locally through newspaper advertisements, pamphlets, and posters. PROMISE participants are at high risk for type 2 diabetes because they were recruited based on the presence of one or more risk factors for type 2 diabetes including obesity, hypertension, a family history of diabetes, and/or a history of gestational diabetes or birth of a macrosomic infant (325).

As part of the screening visit for CANOE, fasting blood samples were collected, and 75-gram oral glucose tolerance tests (OGTTs) were conducted with additional blood samples collected at 30 and 120 minutes for glucose and insulin measurements. Anthropometric measurements were also performed and questionnaires administered. For those who were not eligible for the CANOE trial (because they did not have IGT on the OGTT) or were unwilling to participate, written informed consent was obtained for participation in PROMISE, a longitudinal observational cohort study. At baseline, there were 712 individuals without known diabetes participating in PROMISE. In the second cross-sectional study of this thesis (Chapter 6), those with a glucose concentration in the diabetes range based on the OGTT were excluded (n=58) and so 654 participants were analysed. Participants were contacted annually after the baseline visit to update contact information and collect data on major health events. Participants were invited to return to the clinic examination centers after three years for follow-up assessments. Of the 654 participants at baseline, contact was maintained with n=549 (84%) and 496 (76%) attended the three-year follow-up clinic visits.
4.1 Measures

Fresh fasting, 30 minute and 120 minute blood samples were immediately processed for the determination of serum glucose, and remaining samples were processed and frozen at minus 70°C. Glucose was measured using an enzymatic hexokinase method on the Roche Modular platform. Specific insulin was measured using the Elecsys 1010 immunoassay analyzer (Roche Diagnostics, Basel, Switzerland) and electrochemiluminescence immunoassay. This assay shows 0.05% cross-reactivity to intact human proinsulin and the Des 31,32 circulating split form.

Insulin sensitivity (the inverse of IR) was quantified using the IS\textsubscript{OGTT} index of Matsuda and Defronzo (134), which is defined as $10000 / \sqrt{(FPG \times FPI) \times (G \times I)}$, where FPG = fasting plasma glucose, FPI = fasting plasma insulin, G = mean glucose during the OGTT (calculated from glucose samples at 0, 30 and 120 minutes), and I = mean insulin during the OGTT (calculated from insulin samples at 0, 30 and 120 minutes). This index has been validated against the euglycemic-hyperinsulinemic clamp technique (134). In addition, insulin resistance was measured using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index of Matthews and colleagues (132). HOMA-IR is defined as $FPG \times FPI / 22.5$ (132), and has also been validated against the clamp (132). Beta-cell function was calculated by taking the insulinogenic index divided by HOMA-IR (IGI/IR), which is an established measure that has been used in large studies such as the Diabetes Prevention Program (326). The insulinogenic index is calculated as the ratio of (30 min insulin - fasting insulin) to (30 min glucose - fasting glucose), and has been validated against gold standard measures of insulin secretion (1st phase insulin secretion on intravenous glucose tolerance testing) (144). The Insulin Secretion Sensitivity Index 2 (ISSI-2), which is a more recently proposed measure of beta-cell function that is analogous to the disposition index (DI) but derived from the OGTT (150), was also calculated. This index is defined as the ratio of the area-under-the-insulin curve to the area-under-the-glucose curve, multiplied by the IS\textsubscript{OGTT}, and has been validated against directly measured DI (150).

Vitamin D status, specifically 25-hydroxyvitamin D [25(OH)D], was measured in serum using the DiaSorin “25-OH Vitamin D TOTAL” competitive chemiluminescence
immunoassay on the automated LIAISON® analyzer (Stillwater, MN). This assay has 100% specificity for both 25(OH)D$_2$ and 25(OH)D$_3$. The detection limit of the assay is 10 nmol/L, and the intra- and inter-assay coefficients of variation (CV) were 6.7% and 11.6% respectively, based on the measurements in the baseline blood samples. The 25(OH)D TOTAL method has been validated against the DiaSorin radioimmunoassay (RIA) ($r=0.92$), which is the first test approved for clinical diagnosis by the Food and Drug Administration and which is also the most widely used method (219). In addition, the laboratory in which this assay was conducted participates in the International External Quality Assessment Scheme for Vitamin D Metabolites (DEQAS, Northwest Thames, U.K.), and it has been reported that the 25(OH)D results from this laboratory were consistently within one SD of the group mean in the international DEQAS proficiency surveys (219).

A morning urine sample was also collected for the determination of the albumin/creatinine ratio, a measure of microalbuminuria. Alanine transaminase (ALT) and creatinine were measured in fresh blood samples. ALT, white blood cell count (WBC), and urinary creatinine and albumin were measured using standard laboratory procedures. C-Reactive protein (CRP) was measured using Roche Modular’s particle-enhanced immunoturbidimetric assay, with a minimum detection range of 0.03 mg/L. Estimated glomerular filtration rate (eGFR) was used as a measure of kidney function, calculated using the Modified Diet and Renal Disease (MDRD) formula (327). Analyses for cholesterol, high density lipoprotein (HDL), and triglycerides were performed using Roche Modular’s enzymatic colometric tests (Mississauga, ON). Parathyroid hormone (PTH) was measured using an electrochemiluminescence immunoassay on the Roche Modular E170 analyzer (Laval, QC), which has detection range from 0.127-530 pmol/L.

Adiposity was determined using anthropometric measures including body mass index and waist circumference, and performed with participants in light clothing and with shoes removed. Height and weight were measured to the nearest tenth of a cm and kg, respectively. Each measure was determined twice using standardized procedures, with the average used in the analysis (328). BMI was calculated as weight (kg)/height (m)$^2$. Waist circumference was measured at the natural waist, defined as the narrowest part of the torso, as viewed from behind, or the minimal circumference between the umbilicus and xiphoid process as viewed.
Blood pressure was measured twice in the right arm with the subject seated after 5 minutes resting using an automated sphygmomanometer. Ethnicity, smoking and the participant’s family history of diabetes were assessed using structured questionnaires. Physical activity was determined using a version of the Modifiable Activity Questionnaire (MAQ) (329), which collects information on both leisure and occupational activity over the past year (including measures of frequency and duration). The MAQ has been shown to have good reliability and validity (329). Each reported activity from the MAQ is weighted by its relative intensity, referred to as a MET, thereby deriving MET-hours per week (MET·h·wk\(^{-1}\)) as the final unit of expression. Season was defined using the date participants completed their clinical assessment and was categorized as follows: May-October (summer/early fall); November-April (winter/early spring). Supplement use, including multivitamins, vitamin D, calcium, or vitamin D + calcium, was obtained through an open-ended question on current medication use.

Metabolic syndrome was defined using the recently published harmonized definition (99), and was defined as present if the subject had at least three of the following criteria: elevated waist circumference (≥ 102 cm for men and ≥ 88 cm for women if European-origin; ≥ 90 cm for men and ≥ 80 cm if non-European), elevated triglycerides (≥ 1.7 mmol/L or drug treatment), reduced HDL-C (< 1.0 mmol/L for males and < 1.3 mmol/L in females, or drug treatment), elevated blood pressure (BP) (systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg, or drug treatment), and elevated fasting glucose (≥ 5.6 mmol/L or drug treatment).

4.2 Statistical Analyses

SAS Version 9.1 (version 9.2 for Chapter 8 only) (Cary, NC) was used for all analyses. The normality of the distribution of variables was assessed by the Shapiro-Wilk normality test, in addition to examining histograms and the normal probability plot. Natural logarithmic transformations were applied for all non-normally distributed variables. In descriptive analyses, untransformed continuous variables were reported as mean +/- SD or median with interquartile range in the case of skewed distributions. Categorical variables were reported as n (%). Additional information regarding specific analyses and modelling strategies can be found within the respective papers (Chapters 5-8).
CHAPTER 5:

ASSOCIATION OF VITAMIN D WITH INSULIN RESISTANCE AND BETA-CELL DYSFUNCTION IN SUBJECTS AT RISK FOR TYPE 2 DIABETES

Chapter 5 has been previously published:


Chapter 5: Association of Vitamin D with Insulin Resistance and Beta-cell Dysfunction in Subjects at Risk for Type 2 Diabetes

5.1 Abstract

Objective: To examine cross-sectional associations of serum vitamin D [25-hydroxyvitamin D, 25(OH)D] concentration with insulin resistance (IR) and beta (β)-cell dysfunction in 712 subjects at risk for type 2 diabetes.

Research Design and Methods: Serum 25(OH)D was determined using a chemiluminescence immunoassay. Insulin sensitivity/resistance were measured using the Matsuda insulin sensitivity index for oral glucose tolerance tests (ISOGTT) and homeostasis model assessment of insulin resistance (HOMA-IR). β-cell function was determined using both the insulinogenic index (IGI) divided by HOMA-IR (IGI/IR) and the insulin secretion sensitivity index-2 (ISSI-2).

Results: Linear regression analyses indicated independent associations of 25(OH)D with ISOGTT and HOMA-IR (β=0.004, p=0.0003, and β=-0.003, p=0.0072 respectively), and with IGI/IR and ISSI-2 (β=0.004, p=0.0286, and β=0.003, p=0.0011 respectively) after adjusting for sociodemographics, physical activity, supplement use, parathyroid hormone and BMI.

Conclusions: Vitamin D may play a role in the pathogenesis of type 2 diabetes, as 25(OH)D concentration was independently associated with both insulin sensitivity and β-cell function among individuals at risk of type 2 diabetes.
5.2 Introduction

Emerging evidence suggests a role for vitamin D in the etiology of type 2 diabetes (330). However, associations of vitamin D with insulin resistance (IR) and especially beta (β)-cell dysfunction have been inconsistent (2,23,24,31,58,331). Therefore, our objective was to assess the association of serum vitamin D concentration with IR and β-cell dysfunction in a large, ethnically-diverse North American cohort at risk of type 2 diabetes.

5.3 Methods

5.3.1 Study Design

A detailed methodology for this study has been described previously (328). Briefly, participants in the PROspective Metabolism and ISlet cell Evaluation (PROMISE) cohort were recruited from Toronto and London, Ontario, Canada, from 2004-2006. Participants were 30 years of age and older and at high risk for type 2 diabetes and/or metabolic syndrome (328). The current study includes 712 subjects, 92% of whom were free of diabetes based on oral glucose tolerance tests (OGTT). None had known diabetes at the time of the assessments.

5.3.2 Measures

Fasting blood samples were collected and 75-gram OGTTs were performed. Insulin sensitivity was quantified using the Matsuda insulin sensitivity index for oral glucose tolerance tests (ISOGTT) (134) and IR was measured using the homeostasis model assessment of insulin resistance (HOMA IR) index (132). β-cell dysfunction was determined by dividing the insulinogenic index (IGI) by HOMA-IR (IGI/IR) (144) and by calculating the insulin secretion sensitivity index-2 (ISSI-2) (150).

Serum vitamin D, specifically 25-hydroxyvitamin D [25(OH)D], was measured using DiaSorin’s “25-OH Vitamin D TOTAL” competitive chemiluminescence immunoassay on an automated LIAISON® analyzer (Stillwater, MN). BMI and waist circumference were determined using standardized procedures (328). Parathyroid hormone (PTH) was measured
using an electrochemiluminescence immunoassay on the Roche Modular E170 analyzer (Laval, QC). Structured questionnaires assessed self-reported ethnicity, smoking and physical activity, and included an open-ended question on current medication and supplement use. Season was defined using the participant’s date of clinical assessment, and categorized as May-October (summer/early fall) and November-April (winter/early spring).

5.3.3 Statistical Analysis

SAS Version 9.1 (Cary, NC) was used for all analyses. Natural logarithmic transformations were applied for all non-normally distributed variables. Univariate analyses, including chi-square tests, analysis of variance (ANOVA), and Spearman correlation were conducted to assess the relationship between serum 25(OH)D and potential covariates. Multiple linear regression analyses were conducted to investigate the independent associations of 25(OH)D with measures of insulin sensitivity/resistance (ISOGTT and HOMA-IR) and β-cell dysfunction (IGI/IR and ISSI-2). Model 1 adjusted for sex, age, ethnicity and season; model 2 additionally adjusted for supplement use, total physical activity and PTH; and model 3 additionally adjusted for BMI. Possible effect modifiers were also investigated.

5.4 Results

The sample included 498 (69.9%) females and 462 (64.9%) Caucasians, and the mean age of the participants was 49.6 ± 10.0 years. The mean serum 25(OH)D concentration was 55.81 ± 22.90 nmol/L (range, 10.0 – 161.0). Participant characteristics across quartiles of 25(OH)D concentration, and correlations for continuous variables are presented (Table 5-1). A significant seasonal effect was evident, with higher 25(OH)D concentrations in the summer/early fall (n=343; 59.11 ± 23.71 nmol/L) than in the winter/early spring (n=351; 52.58 ± 21.64 nmol/L) (p=0.0002). Univariate analyses indicated a significant positive association between 25(OH)D and ISOGTT (r=0.30, p<0.0001), a significant negative association between 25(OH)D and HOMA-IR (r=-0.29, p<0.0001), as well as significant positive associations between 25(OH)D and IGI/IR (r=0.14, p=0.0002) and ISSI-2 (r=0.14, p=0.0002).
In multivariate regression analyses, serum 25(OH)D was a significant independent predictor of insulin sensitivity (ISOGTT and HOMA-IR), and β-cell function (IGI/IR and ISSI-2) across all models (Table 5-2). There was a slight attenuation of the association of 25(OH)D on measures of insulin sensitivity and β-cell function after additional adjustment for BMI, but the association remained significant.

We found a significant interaction by BMI, reflecting weaker magnitudes of association of 25(OH)D with measures of insulin sensitivity and β-cell function in obese individuals (BMI ≥ 30 kg/m²) (Table 5-3).

5.5 Conclusions

This study demonstrated independent associations of 25(OH)D with both insulin sensitivity and β-cell function in subjects without known diabetes, the majority of whom were free of diabetes based on OGTTs. The major contribution of this study is the finding of an association between vitamin D and β-cell function. Previous studies assessing the association between 25(OH)D and β-cell function have yielded inconsistent results (2,24,29-31), possibly resulting from small sample sizes, the use of indirect measures of β-cell function (i.e., primarily fasting based measures), and/or the lack of adjustment for background IR. In contrast, our study found a significant positive association between vitamin D and β-cell function, using validated measures of β-cell function which account for the hyperbolic relationship between insulin secretion and insulin sensitivity (150).

Although an inverse association between 25(OH)D and IR has been observed in previous studies (2,24,58,331), the majority of these studies relied primarily on simple fasting-based measures and most did not adjust for physical activity or PTH. In addition, negative findings have been reported, even when more direct measures of insulin sensitivity were used (23,31). Possible reasons for this discrepancy in findings may be due to small sample sizes or differences in study populations. The negative findings of Gulseth et al. (2010) (31) among those with the metabolic syndrome may be attributable to the sequestering of 25(OH)D in adipose tissue (332), resulting in reduced bioavailability of 25(OH)D. Similarly, we found a weaker association of vitamin D with IR and β-cell function in those with a BMI ≥ 30 kg/m².
Strengths of the current study include the measurement of serum 25(OH)D concentration, as well as the use of validated measures of both IR and β-cell dysfunction. In addition, the current study included a large, well-characterized multi-ethnic sample. Limitations include the cross-sectional design, and the lack of “gold standard” measures of IR and β-cell function, which are invasive and costly in large studies. Lastly, 25(OH)D was measured in blood samples obtained across different seasons, although we controlled for a seasonal effect and assessed potential interactions.

In conclusion, vitamin D was significantly related to IR and β-cell function in a multi-ethnic sample at risk for type 2 diabetes. Further research is needed on the prospective association between vitamin D and the underlying disorders of type 2 diabetes in large population-based studies.
Table 5-1. Baseline participant characteristics by 25(OH)D quartiles, PROMISE cohort study

<table>
<thead>
<tr>
<th></th>
<th>Serum 25(OH)D concentration (nmol/L)</th>
<th>Spearman correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q1 (≤ 39.40)</td>
<td></td>
</tr>
<tr>
<td>n (%)</td>
<td>175 (25.22)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>46.86</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Males</td>
<td>52 (24.88)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>123 (25.36)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Caucasian</td>
<td>86 (19.03)</td>
</tr>
<tr>
<td></td>
<td>Hispanic</td>
<td>30 (28.85)</td>
</tr>
<tr>
<td></td>
<td>South Asian</td>
<td>25 (48.08)</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>34 (39.53)</td>
</tr>
<tr>
<td>Smoking (% current)</td>
<td>14 (28.57)</td>
<td></td>
</tr>
<tr>
<td>Anthropometry</td>
<td>BMI (kg/m²)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32.29 (28.84, 37.28)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>31.16 (27.38, 35.31)</td>
<td>-0.25</td>
</tr>
<tr>
<td></td>
<td>29.71 (25.96, 32.86)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>29.10 (25.84, 32.67)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Waist circumference (cm)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>104.05 ± 14.94</td>
<td>-0.21</td>
</tr>
<tr>
<td></td>
<td>99.23 ± 15.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>96.44 ± 15.43</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>95.08 ± 14.72</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Parathyroid Hormone (PTH) (pmol/L)</td>
<td>5.06 ± 2.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>4.76 ± 1.67</td>
<td>-0.20</td>
</tr>
<tr>
<td></td>
<td>4.30 ± 1.57</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>4.23 ± 1.39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Family history of DM (%)</td>
<td>110 (25.52)</td>
<td>0.90</td>
</tr>
<tr>
<td>Total activity (MET-h/wk)</td>
<td>25.87 (8.77, 61.05)</td>
<td>0.0031</td>
</tr>
<tr>
<td></td>
<td>28.62 (10.89, 74.15)</td>
<td>0.15</td>
</tr>
<tr>
<td>Supplement Use</td>
<td>39 (15.42)</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Multi-vitamin (n=265, 42.46%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin D</td>
<td>17 (18.89)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 (14.44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27 (30.00)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33 (36.67)</td>
</tr>
<tr>
<td></td>
<td>p value*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>r value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=93, 14.83%)</td>
<td>Vitamin D + Calcium (n=77, 12.28%)</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 (6.58)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 (16.15)</td>
</tr>
<tr>
<td><strong>Insulin Sensitivity</strong></td>
<td></td>
<td>9.21 (5.91, 14.58)</td>
</tr>
<tr>
<td></td>
<td>HOMA-IR†</td>
<td>2.77 (1.70, 4.26)</td>
</tr>
<tr>
<td><strong>Insulin Resistance</strong></td>
<td></td>
<td>7.22 (4.37, 11.97)</td>
</tr>
<tr>
<td><strong>Beta-cell Function</strong></td>
<td></td>
<td>641.93 (507.39, 803.45)</td>
</tr>
<tr>
<td></td>
<td>ISSI-2‖</td>
<td>690.70</td>
</tr>
</tbody>
</table>

Data are n (%) for categorical variables, mean ± SD for continuous variables, or median (25% & 75% interquartiles) for non-normally distributed variables.

*P values are tests for proportions for categorical variables or tests for equality among quartiles for continuous variables.

†The ISOGTT index is calculated as follows: 10000 / √ (FPG * FPI) * (G * I), where FPG = fasting plasma glucose, FPI = fasting plasma insulin, G = mean glucose during the OGTT, and I = mean insulin during the OGTT (calculated from glucose and insulin samples at 0, 30 and 120 minutes). Glucose and insulin values were in SI units.

‡HOMA-IR is defined as FPG * FPI / 22.5, using SI and metric units for glucose and insulin values respectively

§IGI/IR is calculated as follows: the insulinogenic index ((30 min insulin - fasting insulin) / (30 min glucose - fasting glucose)) divided by HOMA-IR.

‖ISSI-2 is defined as the product of the ratio of total area-under-the-insulin curve to total area-under-the-glucose curve and ISOGTT.
**Table 5-2.** Multiple linear regression analysis for associations of 25(OH)D with measures of insulin sensitivity/resistance and \(\beta\)-cell function

<table>
<thead>
<tr>
<th>Outcome per unit increase in baseline 25(OH)D</th>
<th>Model 1*</th>
<th></th>
<th>Model 2†</th>
<th></th>
<th>Model 3‡</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\beta) (95% CI)</td>
<td>(P) value</td>
<td>(R^2)§</td>
<td>(\beta) (95% CI)</td>
<td>(P) value</td>
<td>(R^2)§</td>
</tr>
<tr>
<td>Insulin Sensitivity/Resistance measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS-OGTT|</td>
<td>0.009 (0.007, 0.011)</td>
<td>&lt;0.0001</td>
<td>0.10</td>
<td>0.008 (0.005, 0.010)</td>
<td>&lt;0.0001</td>
<td>0.15</td>
</tr>
<tr>
<td>HOMA-IR|</td>
<td>-0.010 (-0.012, -0.007)</td>
<td>&lt;0.0001</td>
<td>0.11</td>
<td>-0.008 (-0.010, -0.005)</td>
<td>&lt;0.0001</td>
<td>0.15</td>
</tr>
<tr>
<td>Beta-cell Function measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGI/IR|</td>
<td>0.008 (0.005, 0.011)</td>
<td>&lt;0.0001</td>
<td>0.12</td>
<td>0.007 (0.004, 0.011)</td>
<td>0.0001</td>
<td>0.12</td>
</tr>
<tr>
<td>ISSI-2|</td>
<td>0.005 (0.003, 0.006)</td>
<td>&lt;0.0001</td>
<td>0.15</td>
<td>0.004 (0.003, 0.006)</td>
<td>&lt;0.0001</td>
<td>0.16</td>
</tr>
</tbody>
</table>

* Model 1: adjusted for age, sex, season, ethnicity
† Model 2: adjusted as in model 1 plus supplements, total physical activity, parathyroid hormone
‡ Model 3: adjusted as in model 2 plus BMI
§ \(R^2\) is the coefficient of determination for the model
\| Log transformations
Table 5-3. Association of 25(OH)D with measures of insulin resistance and beta-cell function, stratified by BMI

<table>
<thead>
<tr>
<th>Outcome per unit increase in baseline 25(OH)D</th>
<th>Model 1*</th>
<th>Model 2†</th>
<th>Model 3‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>P value</td>
<td>β (95% CI)</td>
</tr>
<tr>
<td>IS-OGTT§</td>
<td>BMI &lt; 30‖</td>
<td>0.007 (0.004, 0.010)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>BMI ≥ 30¶</td>
<td>0.005 (0.002, 0.008)</td>
<td>0.002</td>
</tr>
<tr>
<td>HOMA-IR§</td>
<td>BMI &lt; 30‖</td>
<td>-0.007 (-0.010, -0.004)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>BMI ≥ 30¶</td>
<td>-0.005 (-0.008, -0.002)</td>
<td>0.003</td>
</tr>
<tr>
<td>IGI/IR§</td>
<td>BMI &lt; 30‖</td>
<td>0.007 (0.003, 0.012)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>BMI ≥ 30¶</td>
<td>0.004 (-0.001, 0.008)</td>
<td>0.10</td>
</tr>
<tr>
<td>ISSI-2§</td>
<td>BMI &lt; 30‖</td>
<td>0.004 (0.002, 0.006)</td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td>BMI ≥ 30¶</td>
<td>0.003 (0.001, 0.005)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

* Model 1: adjusted for age, sex, season, ethnicity  
† Model 2: adjusted as in model 1 plus supplements, total physical activity, parathyroid hormone  
‡ Model 3: adjusted as in model 2 plus BMI  
§ Log transformations  
‖ n=345  
¶ n=367
CHAPTER 6:
ASSOCIATION OF 25(OH)D AND PTH WITH METABOLIC SYNDROME AND ITS
TRADITIONAL AND NONTRADITIONAL COMPONENTS

Chapter 6 has been previously published:


Chapter 6 : Association of 25(OH)D and PTH with Metabolic Syndrome and Its Traditional and Nontraditional Components

6.1 Abstract

Background: Emerging evidence suggests that 25-hydroxyvitamin D [25(OH)D] and PTH may play a role in the etiology of the metabolic syndrome (MetS). However, evidence to date is limited and inconsistent, and few studies have examined associations with nontraditional MetS components.

Objective: The objective of the study was to examine the association of vitamin D and PTH with MetS and its traditional and nontraditional components in a large multi-ethnic sample.

Design, Setting and Participants: In this cross-sectional study, we examined 654 participants from London and Toronto, Ontario, Canada, aged 30 and older with risk factors for type 2 diabetes.

Main Outcome Measures: Presence of MetS and its traditional and nontraditional components was measured.

Results: Approximately 43% of the study participants were classified as having MetS. Higher 25(OH)D was significantly associated with a reduced presence of MetS after adjustment for age, sex, season, ethnicity, supplement use, physical activity, and PTH (OR=0.76, 95% confidence interval 0.62-0.93). PTH was not associated with the presence of MetS after multivariate adjustment. Multivariate linear regression analyses indicated significant adjusted inverse associations of 25(OH)D with waist circumference, triglyceride level, fasting insulin, and alanine transaminase (p<0.041). Elevated PTH was positively associated with waist circumference and high-density lipoprotein cholesterol (p<0.04). Other associations between PTH and MetS components were attenuated after adjustment for adiposity.
Conclusions: Serum 25(OH)D, but not PTH, was significantly associated with the MetS as well as a number of the MetS components after multivariate adjustment. These results suggest that low 25(OH)D may play a role in the etiology of the MetS.
6.2 Introduction

Metabolic syndrome (MetS) refers to a cluster of disorders that indicates increased risk for type 2 diabetes mellitus (type 2 DM) and cardiovascular disease (103). In addition to the traditional or core components of the MetS, which include abdominal obesity, hypertension, and elevated fasting glucose and lipid levels, other disorders including non-alcoholic fatty liver disease (NAFLD) (333), kidney dysfunction (334), subclinical inflammation (335), and hyperinsulinemia (336) are also thought to be part of the cluster. Although progress has been made in identifying risk factors for MetS, including genetic susceptibility (337) and physical inactivity (338), the etiology of MetS is not yet fully understood. Recently low vitamin D, indicated by 25-hydroxyvitamin D [25(OH)D] and elevated PTH levels have emerged as potential risk factors (43,339). Previous literature assessing the association of MetS with vitamin D and PTH has been inconsistent (39,41,43,51-54,339). Whereas a number of previous studies have reported significant associations of low vitamin D and elevated PTH with increased risk of MetS (39,51,52), several studies have also reported no association (41,53,54). Lack of adequate covariate adjustment (e.g. body composition), and differences in the study populations (e.g. some studies were restricted to obese subjects) may partly explain the mixed findings that have been observed. In addition, few previous studies have assessed the role of 25(OH)D or PTH on the nontraditional components of the MetS, including NAFLD, kidney dysfunction, subclinical inflammation, and hyperinsulinemia (52,256,340). Our objective, therefore, was to assess the association of vitamin D and PTH with MetS and its traditional and non-traditional components in a large multi-ethnic sample of subjects at risk for type 2 DM.

6.3 Methods

6.3.1 Study Design

The detailed methodology for this study has been described previously (328). Briefly, participants in the PROspective Metabolism and ISlet cell Evaluation (PROMISE) cohort were recruited from Toronto (43° 40’ N) and London (43° 02’ N), Ontario, Canada, between May 2004 and December 2006. The PROMISE participants are at high risk for type 2 DM.
and/or MetS because they were recruited based on the presence of one or more risk factors for type 2 DM including obesity, hypertension, a family history of diabetes, and/or a history of gestational diabetes or birth of a macrosomic infant. As part of the participant assessment, fasting blood samples were collected, 75-gram oral glucose tolerance tests (OGTTs) and anthropometric measurements performed, and questionnaires administered. Participants were not taking any glucose-lowering medications at the time of the clinic visit. The current study includes 654 subjects who were free of diabetes as confirmed by OGTT, using 1999 WHO criteria (341).

6.3.2 Measures

Fasting blood samples were collected and a 75-g OGTT was then administered, with additional blood samples drawn at 30 min and 2 h for the measurement of glucose and insulin. Normal glucose tolerance and impaired glucose tolerance were classified according to the 1999 WHO criteria (341). A morning urine sample was also collected for the determination of the albumin to creatinine ratio, a measure of microalbuminuria. Serum glucose, alanine transaminase (ALT) and creatinine were measured in fresh samples. Remaining fasting samples were processed and frozen at minus 70°C. Glucose, ALT, white blood cell count (WBC), and urinary creatinine and albumin were measured using standard laboratory procedures. Fasting insulin was measured using the Elecsys 1010 (Roche Diagnostics, Basel, Switzerland) immunoassay analyzer and electrochemiluminescence immunoassay. This assay shows 0.05% cross-reactivity to intact human proinsulin and the Des 31,32 circulating split form. C-Reactive protein (CRP) was measured using Roche Modular’s particle-enhanced immunoturbidimetric assay, with a minimum detection range of 0.03 mg/liter. Analyses for cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides were performed using Roche Modular’s enzymatic colometric tests (Mississauga, ON). Vitamin D status, specifically 25(OH)D, was measured in serum using the DiaSorin vitamin D TOTAL competitive chemiluminescent immunoassay on an automated LIAISON® analyzer (Stillwater, MN). This assay has 100% specificity for both 25(OH)D$_2$ and 25(OH)D$_3$, and has been validated against the DiaSorin RIA (r=0.92) (219), which is the first test approved for clinical diagnosis by the Food and Drug Administration and which is also the most widely used method. The detection limit of the assay is 10
nmol/liter, and the intra- and inter-assay coefficients of variation (CV) were 6.7 and 11.6% respectively. PTH was measured using an electrochemiluminescence immunoassay on the Roche Modular E170 analyzer (Laval, QC), which has detection range from 0.127-530 pmol/liter, and which has been shown to have an intra- and inter-assay CV of 4.1 and 5.8%, respectively (342).

Adiposity was determined using anthropometric measures including body mass index and waist circumference. Each measure was determined twice using standardized procedures, with the average used in the analysis (328). Blood pressure was measured twice in the right arm with the subject seated after 5 min resting using an automated sphygmomanometer. Ethnicity and smoking were assessed using structured questionnaires. Physical activity level was determined using a version of the Modifiable Activity Questionnaire (329), which collects information on both leisure and occupational activity over the past year. Alcohol consumption was assessed with a self-administered multiple-choice questionnaire regarding the number of drinks consumed per week. Season was defined using the date participants completed their clinical assessment and blood collection and was categorized as follows: May through October (summer/early fall); November through April (winter/early spring). Supplement use, including multivitamins, vitamin D, calcium, or vitamin D + calcium, was obtained through an open-ended question on current medication and supplement use.

6.3.2.1 Metabolic Syndrome

6.3.2.1.1 Traditional Components

The recently published harmonized definition of MetS was used (99). MetS was defined as present if the subject had at least three of the following criteria: elevated waist circumference (≥ 102 cm for men and ≥ 88 cm for women if of European-origin; ≥ 90 cm for men and ≥ 80 cm if non-European), elevated triglycerides (≥ 1.7 mmol/L or drug treatment), reduced HDL cholesterol (< 1.0 mmol/L for males and < 1.3 mmol/L in females, or drug treatment), elevated blood pressure (BP) (systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg, or drug treatment), and elevated fasting glucose (≥ 5.6 mmol/L or drug treatment).
6.3.2.1.2 Non-traditional Components

Elevated ALT was used as a surrogate measure for NAFLD (343). Estimated glomerular filtration rate (eGFR) was used as a measure of kidney function, calculated using the Modified Diet and Renal Disease (MDRD) formula (327). The urinary albumin to creatinine ratio was used to define microalbuminuria (344). Elevations in WBC or CRP were considered to indicate the presence of inflammation. The cut points used to dichotomize these nontraditional components were determined based on established clinical guidelines for CRP (>3mg/L), eGFR (< 60 mL/min/1.73m²), and urinary albumin to creatinine ratio (>2.0g/mol for males and >2.8 g/mol for females), for inflammation, kidney dysfunction and microalbuminuria respectively. The lower bound for the upper quartile distribution was used to determine elevated fasting insulin (>104.0 pmol/liter), elevated ALT (>37 U/liter) and elevated WBC (>6.92 x10⁹/liter).

6.3.3 Statistical Analysis

SAS Version 9.1 (SAS Institute, Cary, NC) was used for all analyses. The normality of the distribution of variables was assessed by the Shapiro-Wilk normality test in addition to examining histograms and the normal probability plot. Natural logarithmic transformations were applied for all nonnormally distributed variables. In descriptive analyses, untransformed continuous variables were reported as mean ± SD or median with interquartile range in the case of skewed distributions. Categorical variables were reported as n (%). Spearman correlation analysis was used to assess univariate associations between 25(OH)D and PTH with continuous variables including waist circumference and physical activity. In addition, ANOVAs were used to assess the relationship of 25(OH)D and PTH with potential categorical covariates including age, sex, ethnicity, season, and supplement use. We assessed the association of serum 25(OH)D and PTH with the number of MetS components present using Spearman correlation analysis. For this analysis, 11 MetS components were considered, including abdominal obesity, hypertension, elevated fasting glucose, elevated TG levels, low HDL cholesterol, elevated ALT, WBC, or CRP levels, low eGFR, microalbuminuria, and hyperinsulinemia, definitions of which have been described above. Multivariate logistic regression analyses were conducted to assess the association of
25(OH)D and PTH with the presence of MetS, adjusting for potential confounders. Odds ratios (ORs) are presented to indicate the risk of MetS per standard deviation increase in 25(OH)D or PTH. Possible effect modifiers, specifically age, sex, season and ethnicity, on the associations of 25(OH)D and PTH with MetS were also investigated. Multiple linear regression analyses were then performed to investigate the independent associations of 25(OH)D and PTH with the traditional and non-traditional components of MetS, treated as continuous variables. Staged multivariate modeling was used to construct the models, and covariate adjustment differed slightly for 25(OH)D and PTH analyses due to distinct findings in the univariate analysis, but common covariates included age, sex, season, physical activity and waist circumference.

6.4 Results

Characteristics of the 654 subjects are shown in Table 6-1. Approximately 43% of the study participants were classified as having MetS. The most prevalent MetS component was elevated waist circumference (75.0%), whereas elevated fasting glucose was the least common (13.8%).

There was a significant negative correlation between 25(OH)D and PTH (r=-0.19, p<0.0001) (Table 6-2). As reported in a previous study in the same population (345), we found a significant positive association between serum 25(OH)D levels and age, supplement use, and physical activity, and negative associations of 25(OH)D with BMI and waist circumference. In addition, we found a significant association between 25(OH)D and ethnicity, with Caucasians having significantly higher levels than those of ethnic minorities. In the present study, we found a significant positive association of PTH levels with age, BMI and waist circumference, and a negative association with physical activity (Table 6-2). Vitamin D was significantly associated with season, with higher levels of 25(OH)D in the summer/early fall (25(OH)D=57.37 nmol/liter and 50.48 nmol/liter, p=0.0002 for summer/early fall and winter/early spring respectively).

The associations of serum 25(OH)D and PTH with the number of potential MetS components present are shown in Figure 6-1. Considering all 11 potential components, serum 25(OH)D
decreased as the number of MetS components increased (r=-0.21, p<0.0001), and serum PTH increased as the number of MetS components increased (r=0.15, p=0.0002).

Multivariate associations of 25(OH)D and PTH with the presence of MetS are presented in Figure 6-2. Higher 25(OH)D was significantly associated with a reduced presence of MetS after adjustment for age, sex, season, ethnicity, supplement use, physical activity, and PTH (OR=0.76, 95% confidence interval (CI) 0.62-0.93). Although higher PTH was significantly associated with an increased presence of MetS after adjustment for age, sex, and season, additional adjustment for physical activity and 25(OH)D attenuated the association to non-significance (OR=1.18, 95% CI 0.99-1.42). No significant interaction with age, sex, season or ethnicity was found for the associations of 25(OH)D or PTH with MetS.

Multivariate linear regression analyses for the association of 25(OH)D with both the traditional and nontraditional components of MetS are presented in Table 6-3. In the minimally adjusted model, there were significant inverse associations of 25(OH)D with waist circumference, TGs, diastolic BP, all glucose homeostasis measures, ALT, and inflammatory markers. In addition, there was an initial positive association between 25(OH)D and HDL after adjustment for age, sex, season and ethnicity. Although these associations were attenuated after further adjustment for supplement use, physical activity, PTH, and especially waist circumference, the following remained statistically significant: waist circumference (β =-0.17, p<0.0001), TG level (β =-0.003, p=0.0009), fasting insulin (β=-0.002, p=0.019), and ALT (β=-0.002, p=0.041). In addition, the inverse association between 25(OH)D and ALT remained statistically significant even after adjustment for alcohol consumption (data not shown).

Multivariate linear regression analyses for the associations between PTH and MetS components are presented in Table 6-4. In the minimally adjusted model, there was a significant positive association of PTH with waist circumference, triglycerides, systolic BP, diastolic BP, fasting insulin, and CRP. However, these associations were attenuated with further multivariate adjustment. The only remaining significant association was that between PTH and waist circumference (β=1.92 p<0.0001) after adjustment for age, sex, season, total physical activity, and 25(OH)D. In addition, there was a significant positive association of
PTH with HDL cholesterol (β=0.013, p=0.04) after multivariate adjustment. PTH was not significantly associated with any other traditional or nontraditional component of MetS.

6.5 Discussion

This study demonstrated that 25(OH)D, but not PTH, was significantly associated with MetS in a large multi-ethnic sample. Serum 25(OH)D was also independently associated with a number of MetS components after multivariate adjustment.

While a number of previous studies have found that low 25(OH)D levels were associated with MetS (39,41,43,339), findings have not been consistent (51-54). Studies reporting no association between 25(OH)D and MetS have used indirect measures for 25(OH)D (i.e., dietary vitamin D) (54), or were restricted to unique populations, including elderly (51) or severely obese subjects (52,53). In studies which exclusively examined obese subjects, it may be that vitamin D is less bioavailable in these subjects due to the sequestering of 25(OH)D in adipose tissue (346).

Regarding the traditional components of MetS, our study revealed that 25(OH)D was negatively associated with triglyceride level and waist circumference, a finding which is consistent with the majority of previous research (43,52,339). A possible mechanism by which 25(OH)D may be associated with TG levels is through increased activity of lipoprotein lipase, which has been shown to be regulated by vitamin D in adipocytes (347). Of the nontraditional MetS components, 25(OH)D was also found to have a significant inverse association with fasting insulin, which is supported by previous findings suggesting an association of 25(OH)D with insulin resistance and type 2 DM (345,348). Lower 25(OH)D was also significantly associated with elevated ALT, suggesting greater amounts of hepatic fat. To our knowledge, no previous study has examined the association between 25(OH)D and ALT, which is a marker of NAFLD (343), an emerging component of the MetS cluster (333). However, Targher et al. (2007) found that NAFLD patients had significantly lower 25(OH)D concentrations than matched controls, and that 25(OH)D concentrations were inversely associated with severity of liver histology (349). It is possible that these findings are due to an unhealthy lifestyle or to the close association between NAFLD and other components of the MetS (e.g. obesity). However, the present study found a significant
inverse association between 25(OH)D and ALT independent of alcohol consumption, physical activity, and abdominal obesity. In addition, given that vitamin D is converted to 25(OH)D in the liver, it is also possible that abnormalities in vitamin D metabolism may be a result of NAFLD. However, previous studies have reported that vitamin D metabolites and their binding protein may be impaired only in NAFLD patients with severe hepatic dysfunction (350). In our study, the majority of participants were within the normal range of ALT and none were known to have severe cirrhosis.

The initial significant association between 25(OH)D and WBC was significant after adjustment for age, sex, season, ethnicity, supplement use, physical activity and PTH. However, this association was attenuated after further adjustment for waist circumference, suggesting that this association is largely explained by obesity. Although it has been reported that 25(OH)D may have anti-inflammatory effects (351), previous studies have found no association between 25(OH)D and inflammatory markers, particularly CRP (266,352). Given these inconsistencies, further research in this area is needed.

Although we found a significant correlation between serum PTH and the number of MetS components present, our multivariate analyses did not indicate a significant independent association of PTH with MetS, or with the majority of the MetS components. The few studies that have assessed the association between PTH and MetS have reported mixed results (39,41,43,51-53). Previous reports of a positive association between PTH and MetS have been reported in populations restricted to morbidly obese (52), or elderly subjects (51), and in one case adiposity was not taken into account (39). In the current study, however, we did find a significant positive association between PTH and waist circumference, which has been reported in previous studies (43,353). These findings are supported by evidence suggesting increased weight gain and lipogenesis with elevated PTH (354). Of particular interest as well is the initial significant positive association between PTH and BP, which was attenuated and became non-significant after adjustment for waist circumference. Previous studies have reported a positive association between serum PTH and blood pressure (43,52,353). However, our findings suggest that the association between PTH and blood pressure is mediated by adiposity. Other studies have also reported no association between PTH and blood pressure (39,51,53). We also found a stronger positive association of blood pressure
with waist circumference, than with PTH, which may partly explain such findings. Given current inconsistencies in the literature, further research in this area is needed. In addition, initial significant associations of PTH with fasting insulin and CRP were also largely attenuated by adjustment for waist circumference, indicating that the association between PTH and these MetS components was largely mediated by the positive association between PTH and body composition. However, our results indicated a significant positive association of PTH with HDL cholesterol even after multivariate adjustment. Possible reasons for this paradoxical finding are unknown and should be explored in further studies.

Some limitations of the present study must be highlighted. Given that this study was cross-sectional in nature, temporal associations cannot be determined. In addition, results of this study cannot be considered representative of the general population because study subjects were recruited based on being at risk for type 2 DM. As such, we were not able to assess whether 25(OH)D or PTH levels were different among those at risk of type 2 diabetes compared to those not at risk, due to the lack of a comparison group in the cohort consisting of those free of all diabetes risk factors. We also did not measure additional markers of inflammation, including TNF-α and IL-6, which may have provided a more accurate assessment of the inflammatory state in MetS. In addition, although controversies exist regarding the clinical utility of the MetS concept (102), it has continued to be a useful construct in understanding the clustering of metabolic disorders and the association of this cluster with significant increased risks of type 2 DM and cardiovascular disease (103).

Strengths of this study include the large multiethnic cohort and the assessment of a number of nontraditional components of MetS.

In conclusion, the present study found a significant inverse association between 25(OH)D and MetS. Serum 25(OH)D was also independently associated with various traditional and nontraditional components of MetS. There was no association between PTH and MetS, and the association of PTH with various components of the MetS appeared to be largely mediated by body composition. Further research, particularly prospective studies, are needed to verify our findings.
Table 6-1. Baseline participant characteristics, PROMISE cohort study, 2004-2006

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>654</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49.10 ± 9.80</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>461 (70.49)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>n (%) Caucasian</td>
<td>422 (64.53)</td>
</tr>
<tr>
<td>n (%) Hispanic</td>
<td>104 (15.90)</td>
</tr>
<tr>
<td>n (%) South Asian</td>
<td>44 (6.73)</td>
</tr>
<tr>
<td>n (%) other</td>
<td>84 (12.84)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.09 ± 6.33</td>
</tr>
<tr>
<td>Natural waist circumference (cm)</td>
<td>98.39 ± 15.56</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>56.41 ± 23.17</td>
</tr>
<tr>
<td>Parathyroid hormone (pmol/L)</td>
<td>4.56 ± 1.70</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>126.05 ± 16.16</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80.20 ± 10.51</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.30 (0.96, 1.88)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.30 (1.1, 1.6)</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT) (units/L)</td>
<td>27.00 (21.00, 36.00)</td>
</tr>
<tr>
<td>Albumin:creatinine ratio (g/mol)</td>
<td>0.54 (0.37, 0.90)</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate (mL/min/1.73 m²)</td>
<td>93.59 ± 19.44</td>
</tr>
<tr>
<td>White blood cell count (x10³/L)</td>
<td>6.02 ± 1.57</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>2.05 (1.0, 4.7)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>4.90 (4.6, 5.2)</td>
</tr>
<tr>
<td>Metabolic Syndrome (%yes)</td>
<td>279 (42.66)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Elevated waist circumference(^a) (%yes)</td>
<td>489 (74.77)</td>
</tr>
<tr>
<td>Elevated Triglycerides(^b) (%yes)</td>
<td>258 (39.45)</td>
</tr>
<tr>
<td>Low HDL(^c) (%yes)</td>
<td>300 (45.87)</td>
</tr>
<tr>
<td>High blood pressure(^d) (%yes)</td>
<td>359 (54.89)</td>
</tr>
<tr>
<td>Elevated fasting glucose(^e) (%yes)</td>
<td>90 (13.76)</td>
</tr>
</tbody>
</table>

Data are mean ± SD for normally distributed continuous variables, or median (25% & 75% interquartiles) for non-normally distributed continuous variables, unless otherwise stated.

\(^a\)Elevated waist circumference: ≥ 102 cm for men and ≥ 88 cm for women if European-origin; ≥ 90 cm for men and ≥ 80 cm for women if non-European

\(^b\)Elevated Triglycerides ≥ 1.7 mmol/L (or drug treatment)

\(^c\)Low HDL < 1.0 mmol/L for males, < 1.3 mmol/L for females (or drug treatment)

\(^d\)High blood pressure (systBP ≥ 130 and/or diastBP ≥ 85 mmHg or drug treatment)

\(^e\)Elevated fasting glucose (≥ 5.6 mmol/L or drug treatment)
Table 6-2. Correlation analysis of 25(OH)D and PTH with metabolic and anthropometric variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>25(OH)D</th>
<th>PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D</td>
<td>1.00</td>
<td>-0.19 (&lt;0.0001)</td>
</tr>
<tr>
<td>PTH</td>
<td>-0.19 (&lt;0.0001)</td>
<td>1.00</td>
</tr>
<tr>
<td>Age</td>
<td>0.18 (&lt;0.0001)</td>
<td>0.10 (0.013)</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.25 (&lt;0.0001)</td>
<td>0.25 (&lt;0.0001)</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>-0.21 (&lt;0.0001)</td>
<td>0.24 (&lt;0.0001)</td>
</tr>
<tr>
<td>Physical activity</td>
<td>0.18 (&lt;0.0001)</td>
<td>-0.11 (0.007)</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>-0.01 (0.71)</td>
<td>0.14 (0.0006)</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>-0.06 (0.11)</td>
<td>0.17 (&lt;0.0001)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.21 (&lt;0.0001)</td>
<td>0.05 (0.18)</td>
</tr>
<tr>
<td>HDL</td>
<td>0.15 (0.0002)</td>
<td>0.01 (0.82)</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>-0.09 (0.0248)</td>
<td>0.01 (0.78)</td>
</tr>
<tr>
<td>Albumin:creatinine ratio</td>
<td>-0.06 (0.15)</td>
<td>0.04 (0.31)</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate</td>
<td>-0.17 (&lt;0.0001)</td>
<td>-0.02 (0.56)</td>
</tr>
<tr>
<td>White blood cell count</td>
<td>-0.18 (&lt;0.0001)</td>
<td>0.01 (0.90)</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>-0.07 (0.08)</td>
<td>0.14 (0.0004)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>-0.03 (0.40)</td>
<td>0.10 (0.0132)</td>
</tr>
<tr>
<td>2-hour glucose (mmol/L)</td>
<td>-0.06 (0.14)</td>
<td>0.11 (0.008)</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>-0.31 (&lt;0.0001)</td>
<td>0.19 (&lt;0.0001)</td>
</tr>
</tbody>
</table>

Data are Spearman correlation coefficients (r) with corresponding p values in brackets
Figure 6-1. Box plots of serum 25(OH)D (A) and serum PTH (B) levels by number of metabolic syndrome components.

Each box displays the median, upper and lower quartiles of the respective distribution. Box whiskers represent the maximum and minimum range, with outliers (shown as dots) representing those outside the 10th and 90th percentile.
**Figure 6-2.** Multivariate logistic regression analysis of associations of 25(OH)D and PTH with prevalence of the metabolic syndrome.

Note: The Odds Ratio indicates the odds of having the metabolic syndrome for each standard deviation change in 25(OH)D (Models 1a and 1b) and PTH (Models 2a and 2b).

Association between 25(OH)D and MetS: adjusted for age, sex, season, ethnicity (Model 1a) and adjusted as in model 1a plus supplements, physical activity, PTH (Model 1b).

Association between PTH and MetS: adjusted for age, sex, season (Model 2a) and adjusted as in model 2a plus physical activity, vitamin D (Model 2b).
Table 6-3. Multivariate linear regression analysis of associations of 25(OH)D with metabolic syndrome and its traditional & non-traditional components

<table>
<thead>
<tr>
<th>Outcome per unit increase in baseline 25(OH)D</th>
<th>Model 1 (n=636)</th>
<th></th>
<th>Model 2 (n=594)</th>
<th></th>
<th>Model 3 (n=594)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$ (95% CI)</td>
<td>$P$ value</td>
<td>$\beta$ (95% CI)</td>
<td>$P$ value</td>
<td>$\beta$ (95% CI)</td>
<td>$P$ value</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>-0.223 (-0.27, -0.17)</td>
<td>&lt;0.0001</td>
<td>-0.17 (-0.23, -0.12)</td>
<td>&lt;0.0001</td>
<td>-0.003 (-0.005, -0.001)</td>
<td>0.0009</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.006 (-0.007, -0.004)</td>
<td>&lt;0.0001</td>
<td>-0.005 (-0.007, -0.003)</td>
<td>&lt;0.0001</td>
<td>-0.003 (-0.005, -0.001)</td>
<td>0.48</td>
</tr>
<tr>
<td>HDL</td>
<td>0.002 (0.001, 0.003)</td>
<td>0.0002</td>
<td>0.001 (0.0004, 0.002)</td>
<td>0.006</td>
<td>0.0003 (0.001, 0.001)</td>
<td>0.64</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>-0.05 (-0.10, -0.002)</td>
<td>0.058</td>
<td>-0.018 (-0.07, 0.04)</td>
<td>0.53</td>
<td>0.020 (-0.04, 0.08)</td>
<td>0.48</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>-0.05 (-0.09, -0.01)</td>
<td>0.0065</td>
<td>-0.03 (-0.07, 0.01)</td>
<td>0.18</td>
<td>0.009 (-0.03, 0.05)</td>
<td>0.76</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>-0.0005 (-0.001, -0.0001)</td>
<td>0.011</td>
<td>-0.0004 (-0.001, 0.0001)</td>
<td>0.06</td>
<td>-0.0001 (-0.0005, 0.0003)</td>
<td>0.94</td>
</tr>
<tr>
<td>2-hour glucose (mmol/L)</td>
<td>-0.001 (-0.002, -0.0001)</td>
<td>0.037</td>
<td>-0.001 (-0.001, 0.0004)</td>
<td>0.29</td>
<td>-0.00004 (-0.001, 0.001)</td>
<td>0.019</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>-0.009 (-0.011, -0.007)</td>
<td>&lt;0.0001</td>
<td>-0.007 (-0.01, -0.004)</td>
<td>&lt;0.0001</td>
<td>-0.002 (-0.005, -0.0004)</td>
<td>0.041</td>
</tr>
<tr>
<td>Alanine aminotransferase (units/L)</td>
<td>-0.002 (-0.004, -0.001)</td>
<td>0.0019</td>
<td>-0.002 (-0.004, -0.001)</td>
<td>0.0060</td>
<td>-0.002 (-0.003, -0.0001)</td>
<td>0.23</td>
</tr>
<tr>
<td>White blood cell count (x10$^9$/L)</td>
<td>-0.011 (-0.02, -0.006)</td>
<td>&lt;0.0001</td>
<td>-0.008 (-0.01, -0.002)</td>
<td>0.0069</td>
<td>-0.004 (-0.010, 0.002)</td>
<td>0.08</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>-0.006 (-0.010, -0.001)</td>
<td>0.0018</td>
<td>-0.002 (-0.006, -0.002)</td>
<td>0.27</td>
<td>0.003 (-0.0004, -0.0001)</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>-0.002)</td>
<td>0.002)</td>
<td>0.007)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------------------</td>
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<td>------------</td>
<td>------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin:creatinine ratio (g/mol)</td>
<td>-0.003 (-0.006, 0.001)</td>
<td>0.12</td>
<td>-0.003 (-0.006, 0.001)</td>
<td>0.11</td>
<td>-0.003 (-0.007, 0.001)</td>
<td>0.13</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate (mL/min/1.73 m²)</td>
<td>-0.05 (-0.11, 0.019)</td>
<td>0.17</td>
<td>-0.08 (-0.15, -0.008)</td>
<td>0.029</td>
<td>-0.07 (-0.14, 0.01)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age, sex, season, ethnicity
Model 2: adjusted as in model 1 plus supplements, physical activity, PTH
Model 3: adjusted as in model 2 plus waist circumference
Table 6-4. Multivariate linear regression analysis of associations of PTH with metabolic syndrome, and its traditional & non-traditional components

<table>
<thead>
<tr>
<th>Outcome per unit increase in baseline 25(OH)D</th>
<th>Model 1 (n=630)</th>
<th>Model 2 (n=594)</th>
<th>Model 3 (n=594)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI) P value</td>
<td>β (95% CI) P value</td>
<td>β (95% CI) P value</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>2.21 (1.54, 2.89) &lt;0.0001</td>
<td>1.92 (1.22, 2.61) &lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.02 (0.003, 0.046) 0.026</td>
<td>0.012 (-0.011, 0.034) 0.30</td>
<td>-0.008 (-0.030, 0.014) 0.48</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.002 (-0.014, 0.009) 0.68</td>
<td>0.003 (-0.009, 0.016) 0.59</td>
<td>0.013 (0.001, 0.025) 0.040</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.73 (0.07, 1.39) 0.030</td>
<td>0.77 (0.07, 1.46) 0.031</td>
<td>0.25 (-0.43, 0.94) 0.47</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.77 (0.30, 1.24) 0.0014</td>
<td>0.71 (0.21, 1.21) 0.0052</td>
<td>0.24 (-0.24, 0.72) 0.33</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.004 (-0.0004, 0.009) 0.075</td>
<td>0.004 (-0.001, 0.009) 0.12</td>
<td>0.0002 (-0.005, 0.005) 0.94</td>
</tr>
<tr>
<td>2-hour glucose (mmol/L)</td>
<td>0.010 (-0.002, 0.022) 0.092</td>
<td>0.008 (-0.004, 0.02) 0.20</td>
<td>0.002 (-0.01, 0.01) 0.76</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>0.08 (0.05, 0.11) &lt;0.0001</td>
<td>0.06 (0.03, 0.08) 0.003</td>
<td>-0.011 (-0.015, 0.04) 0.41</td>
</tr>
<tr>
<td>Alanine aminotransferase (units/L)</td>
<td>0.008 (-0.012, 0.027) 0.43</td>
<td>0.005 (-0.015, 0.026) 0.60</td>
<td>-0.0005 (-0.021, 0.020) 0.96</td>
</tr>
<tr>
<td>White blood cell count (x10^9/L)</td>
<td>0.03 (-0.04, 0.10) 0.42</td>
<td>-0.006 (-0.07, 0.08) 0.88</td>
<td>-0.04 (-0.12, 0.03) 0.25</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>0.07 (0.02, 0.006) 0.071</td>
<td>0.006 0.006</td>
<td>0.010 0.68</td>
</tr>
<tr>
<td>(mg/L)</td>
<td>0.12</td>
<td>(0.02, 0.12)</td>
<td>(-0.04, 0.06)</td>
</tr>
<tr>
<td>------------------</td>
<td>------</td>
<td>--------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Albumin:creatinine ratio (g/mol)</td>
<td>0.026 (-0.016, 0.07)</td>
<td>0.22</td>
<td>0.015 (-0.03, 0.06)</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate (mL/min/1.73 m²)</td>
<td>-0.20 (-1.06, 0.65)</td>
<td>0.64</td>
<td>-0.57 (-1.45, 0.31)</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age, sex, season
Model 2: adjusted as in model 1 plus physical activity, 25(OH)D
Model 3: adjusted as in model 2 plus waist circumference
CHAPTER 7:

PROSPECTIVE ASSOCIATIONS OF VITAMIN D WITH BETA-CELL FUNCTION AND GLYCEMIA: THE PROMISE (PROSPECTIVE METABOLISM AND ISLET CELL EVALUATION) COHORT STUDY

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Chapter 7: Prospective Associations of Vitamin D with Beta-Cell Function and Glycemia: the PROMISE (PROspective Metabolism and ISlet cell Evaluation) Cohort Study

7.1 Abstract

Objective: To examine the prospective associations of baseline vitamin D [25-hydroxyvitamin D, 25(OH)D] with insulin resistance (IR), \( \beta \)-cell function, and glucose homeostasis in subjects at risk for type 2 diabetes.

Research Design and Methods: We followed 489 individuals, aged 50 ± 10 years, for 3 years. At baseline and follow-up, 75g oral glucose tolerance tests (OGTTs) were administered. Using validated proxy measures, IR was measured using the Matsuda index (IS\textsubscript{OGTT}) and the homeostasis model assessment of IR (HOMA-IR), \( \beta \)-cell function was determined using both the insulinogenic index divided by HOMA-IR (IGI/IR) and the insulin secretion sensitivity index-2 (ISSI-2), and glycemia was assessed using the area under the curve of glucose (AUC\textsubscript{glucose}) during the OGTT. Regression models were adjusted for age, sex, ethnicity, season, and baseline value of the outcome variable, as well as baseline and change in physical activity, vitamin D supplement use and BMI.

Results: Multivariate linear regression analyses indicated no significant association of baseline 25(OH)D with follow-up IS\textsubscript{OGTT} or HOMA-IR. There were, however, significant positive associations of baseline 25(OH)D with follow-up IGI/IR (\( \beta=0.005, p=0.015 \)) and ISSI-2 (\( \beta=0.002, p=0.023 \)), and a significant inverse association of baseline 25(OH)D with follow-up AUC\textsubscript{glucose} (\( \beta=-0.001, p=0.007 \)). Progression to dysglycemia (impaired fasting glucose, impaired glucose tolerance, or type 2 diabetes) occurred in 116 subjects. Logistic regression analyses indicated a significant reduced risk of progression with higher baseline 25(OH)D (adjusted odds ratio 0.69 [95% CI 0.53-0.89]), but this association was not significant after further adjustment for baseline and change in BMI (0.78 [95% CI 0.59-1.02]).
**Conclusions:** Higher baseline 25(OH)D independently predicted better β-cell function and lower AUC\textsubscript{glucose} at follow-up, supporting a potential role for vitamin D in type 2 diabetes etiology.
7.2 Introduction

Emerging evidence suggests that vitamin D [25-hydroxyvitamin D; 25(OH)D] may play a role in the etiology of type 2 diabetes (355,356). Vitamin D levels are lower in those with type 2 diabetes and impaired glucose tolerance (IGT) compared with those with normal glucose tolerance (NGT) (3,5,6). In addition, most (8-11), but not all (17,18), prospective studies have shown a significant inverse association of baseline serum 25(OH)D with incident diabetes. To date, however, the exact mechanisms/disorders through which vitamin D affects diabetes risk is not yet fully known, particularly whether vitamin D plays a role in insulin resistance (IR) and/or β-cell dysfunction, the main pathophysiological disorders underlying type 2 diabetes.

Previous studies have reported significant inverse associations of vitamin D with IR (2,24-26,357) and β-cell dysfunction (29,30), including our cross-sectional study in the current cohort (345), although findings have been inconsistent (2,23,24,31,32). These inconsistencies may be attributed to the cross-sectional design of these studies or the use of less precise measures of outcomes. Only two prospective studies have been conducted to date, both of which reported significant inverse associations of baseline 25(OH)D with IR after 5 and 10 years of follow-up, respectively, in largely Caucasian cohorts (11,58). No study has yet examined β-cell function prospectively in relation to vitamin D. The objective of this study, therefore, was to examine the prospective association of baseline serum 25(OH)D with IR, β-cell function and glucose homeostasis in a cohort of 489 subjects at high risk of type 2 diabetes.

7.3 Methods

7.3.1 Study Design

A detailed methodology of the Prospective Metabolism and Islet cell Evaluation (PROMISE) Cohort Study has been published previously (328,345). PROMISE participants, aged ≥ 30 years, were recruited from Toronto and London, Ontario, Canada, between May 2004 and December 2006. Participants were at high risk for type 2 diabetes as they were recruited based on the presence of one or more risk factors for diabetes including obesity,
hypertension, a family history of diabetes and/or a history of gestational diabetes or birth of a macrosomic infant (328). Participants were contacted annually after the baseline visit to update contact information and collect data on major health events. Participants were invited to return to the clinic examination centers after 3 years for follow-up assessments.

7.3.2 Participants

At baseline, 654 individuals without diabetes participated; the mean age was 50.19 ± 9.67 years, 357 (73.01%) were female, and 142 (29%) reported non-Caucasian ethnicity (12% Hispanic, 7% South Asian and 10% other). Of these 654 participants, contact was maintained with n=549 (84%) and 496 (76%) attended the three-year follow-up clinic visits. Those who attended the follow-up examination were more likely to be older, female and Caucasian (p<0.02) than those who did not attend, but there were no significant differences in BMI or measures of IR, β-cell function or glucose homeostasis (p≥0.07).

7.3.3 Measures

As part of the baseline and 3-year clinic assessment, fasting blood samples were collected and 75-gram oral glucose tolerance tests (OGTTs) were conducted with additional blood samples collected at 30 and 120 min for glucose and insulin measurements. Fresh fasting, 30- and 120-min blood samples were immediately processed for the determination of serum glucose, and remaining samples were processed and frozen at -70°C. Glucose was measured using an enzymatic hexokinase method on the Roche Modular platform. Specific insulin was measured using the Elecsys 1010 immunoassay analyzer (Roche Diagnostics, Basel, Switzerland) and electrochemiluminescence immunoassay. This assay shows 0.05% cross-reactivity to intact human proinsulin and the Des 31,32 circulating split form. Parathyroid hormone (PTH) was measured using an electrochemiluminescence immunoassay on the Roche Modular E170 analyzer (Laval, QC), which has a detection range from 0.127 to 530 pmol/L. Baseline C-reactive protein (CRP) was also measured in fasting samples using Roche Modular’s particle-enhanced immunoturbidimetric assay, with a minimum detection range of 0.03 mg/L.
Vitamin D status, specifically 25(OH)D, was measured in serum using the DiaSorin “25 OH Vitamin D TOTAL” competitive chemiluminescent immunoassay on the automated LIAISON® analyzer (Stillwater, MN). This assay has 100% specificity for both 25(OH)D$_2$ and 25(OH)D$_3$. The detection limit of the assay is 10 nmol/L, and we found that it has an intra-assay coefficient of variation of 6.7% and an inter-assay coefficient of variation of 11.6%. The 25(OH)D TOTAL method has been validated against the DiaSorin radioimmunoassay ($r=0.92$), which is the first test approved for clinical diagnosis by the Food and Drug Administration and which is also the most widely used method (219). In addition, the laboratory in which this assay was conducted participates in the International External Quality Assessment Scheme for Vitamin D Metabolites (DEQAS, Northwest Thames, U.K.), and it has been reported that the 25(OH)D results from this laboratory were consistently within one SD of the group mean in the international DEQAS proficiency surveys (219).

Anthropometric measurements were performed with participants in light clothing and with shoes removed. Height and weight were measured to the nearest tenth of a cm and kg, respectively, and BMI was calculated as weight in kilograms divided by the square of height in meters ($kg/m^2$). Waist circumference was measured at the natural waist, defined as the narrowest part of the torso, as viewed from behind, or the minimal circumference between the umbilicus and xiphoid process, as viewed from the front. Blood pressure was measured twice in the right arm with the subject seated after a 5-min rest using an automated sphygmomanometer. Each measure was determined twice using standardized procedures, with the average used in the analysis. Physical activity level was determined using a version of the Modifiable Activity Questionnaire (MAQ) (329), which collects information on both leisure and occupational activity over the past year (including measures of frequency and duration). The MAQ has been shown to have good reliability and validity (329). Each reported activity from the MAQ is weighted by its relative intensity, referred to as a metabolic equivalent of task (MET), thereby deriving MET hours per week (MET h/week) as the final unit of expression. Season was defined using the date participants completed their baseline assessment and was categorized as follows: May through October (summer/early fall); November through April (winter/early spring). Supplement use, specifically any vitamin or multivitamin containing vitamin D, was obtained through an open-ended question.
on current medication use. Ethnicity, smoking and the participant’s family history of diabetes were assessed using structured questionnaires.

7.3.3.1 Outcome Variables

IR was quantified using both the IS\textsubscript{OGTT} index of Matsuda and Defronzo (134) and the homeostasis model assessment of IR (HOMA-IR) index of Matthews and colleagues (132). The IS\textsubscript{OGTT} index, which is a measure of insulin sensitivity (134), is defined as $10,000 / \sqrt{(FPG \times FPI) \times (G \times I)}$, where FPG refers to fasting plasma glucose, FPI refers to fasting plasma insulin, G refers to mean glucose during the OGTT, and I refers to mean insulin during the OGTT. This index reflects whole body insulin sensitivity, and has been validated against the euglycemic-hyperinsulinemic clamp technique (134). HOMA-IR, which is defined as $FPG \times FPI / 22.5$ (132), largely reflects hepatic IR, and has also been validated against the clamp (132). β-cell dysfunction was calculated by taking the insulinogenic index (IGI) divided by HOMA-IR (IGI/IR) (144), which is a widely used measure of β-cell function. The insulinogenic index is calculated by taking the ratio of (30 min insulin minus fasting insulin) to (30 min glucose minus fasting glucose) (144), and has been validated against gold standard measures of insulin secretion (first phase insulin secretion on intravenous glucose tolerance testing [IVGTT]). The insulin secretion sensitivity index-2 (ISSI-2), which is a more recently proposed measure of β-cell function that is analogous to the disposition index but derived from the OGTT (149), was also calculated. This index, which has been validated against directly measured disposition index (150), is defined as the ratio of the area under the insulin curve (AUC\textsubscript{insulin}) to the area under the glucose curve (AUC\textsubscript{glucose}), multiplied by IS\textsubscript{OGTT} (150). Glycemia was assessed using the AUC\textsubscript{glucose} during the OGTT, calculated using the trapezoidal rule. Progression to dysglycemia was defined as having developed impaired fasting glucose (IFG), IGT or incident type 2 diabetes based on the OGTT at the 3-year follow-up. IFG, IGT and diabetes were categorized using 1999 World Health Organization criteria (341). In addition, participants who reported being diagnosed with type 2 diabetes between baseline and follow-up during either the annual telephone contacts or the clinical examinations also were considered to have incident diabetes at follow-up. Verification of self-reported diabetes was obtained from the
participant’s physician through a supplementary form requesting information on the date of diagnosis, blood glucose levels supporting the diagnosis, and current treatment.

7.3.4 Statistical Analysis

SAS version 9.1 was used for all analyses. Continuous variables were reported as means ± SD or median (interquartile range) in the case of skewed distributions, whereas categorical variables were reported as n (%). Natural logarithmic transformations were applied for all non-normally distributed variables. Chi-square tests and Student t-tests were used to examine differences between those who did and did not attend the 3-year follow-up examination. Changes in participant characteristics between baseline and follow-up were tested using the McNemar test for categorical variables and the paired Student t-test or Wilcoxon signed-rank test for normally-distributed or non-normally distributed continuous variables, respectively.

Percentage change was calculated as the follow-up value minus baseline value divided by the baseline value, multiplied by 100. Multiple linear regression analyses were conducted to investigate the association of baseline serum 25(OH)D as the independent variable with measures of IR (ISOGTT and HOMA-IR), β-cell function (IGI/IR and ISSI-2), and glycemia (AUCglucose) at the 3-year follow-up as the dependent variables. Separate models were used for each outcome variable, which included adjustment for the baseline value of the outcome measure being assessed. Differences in baseline serum 25(OH)D according to glycemic progression status (NGT vs. IFG, IGT or type 2 diabetes at follow-up) were evaluated using t tests. The association of baseline serum 25(OH)D with progression to dysglycemia was assessed using multivariate logistic regression analysis. Odds ratios are presented to indicate the risk of progression to dysglycemia per SD increase in 25(OH)D. Potential confounders were identified on the basis of the results of previous cross-sectional analyses in the PROMISE study cohort (345). Significant positive associations of baseline serum 25(OH)D levels with age, vitamin D supplement use, and physical activity, and negative associations of 25(OH)D with BMI and waist circumference were documented. On the basis of these findings, staged multivariate regression models were constructed for the current analysis. Model 1 was adjusted for age, sex, ethnicity, and season of the 25(OH)D measurement; model 2 was additionally adjusted for baseline and change in physical activity and vitamin D.
supplement use; and model 3 was additionally adjusted for baseline and change in BMI. Possible effect modifiers including sex, ethnicity, BMI and season also were investigated.

7.4 Results

Baseline vitamin D measurements were available for 489 (99%) of the 496 participants who came back for their follow-up visit. Of these 489 participants, 116 individuals (23.72%) progressed to dysglycemia, of which 11 (2.25%) had IFG, 75 (15.34%) had IGT, and 30 (6.13%) were classified as having type 2 diabetes. Of 30 participants with diabetes at follow-up, 6 individuals were diagnosed between baseline and follow-up and the remaining 24 were classified as having diabetes on the basis of the OGTT at the 3-year clinic assessment. Participant characteristics at baseline and at follow-up, as well as percentage change in these characteristics over the follow-up period are presented in Table 7-1. The mean baseline serum 25(OH)D concentration was 58.01 ± 23.26 nmol/L. Based on the Institute of Medicine’s 2011 Dietary Reference Intakes for Vitamin D (228), we found that ~11, 22 and 63% of our cohort had deficient (<30 nmol/L), insufficient (<40 nmol/L), and sufficient (≥50 nmol/L) 25(OH)D levels. Overall, participants gained weight over the 3 years and also reported significantly higher vitamin D supplement use at follow-up. In addition, the significant decrease in ISOGTT and significant increase in HOMA-IR over the 3 years indicate worsening IR. There was also a significant decrease in both IGI/IR and ISSI-2 and a significant increase in AUCglucose, indicating that participants also had deteriorating β-cell function and glucose homeostasis over the follow-up period.

In the multivariate linear regression analyses (Table 7-2), baseline serum 25(OH)D was not significantly associated with follow-up ISOGTT in model 1 (β=0.002, p=0.12), and remained nonsignificant with additional covariate adjustment. Although an initial significant inverse association of baseline 25(OH)D with follow-up HOMA-IR was observed after adjustment for age, sex, ethnicity, season of the 25(OH)D measurement, baseline HOMA-IR, and baseline and change in physical activity and supplement use, these findings were attenuated to nonsignificance after additional adjustment for baseline and change in BMI (β=−0.001, p=0.32). In contrast, there was a significant positive association of baseline serum 25(OH)D with both measures of β-cell function at follow-up (β=0.005, p=0.015 and β=0.002, p=0.023
for IGI/IR and ISSI-2, respectively). There was also a significant inverse association of baseline 25(OH)D with follow-up AUC\textsubscript{glucose} (β=-0.001, p=0.007). Additional adjustment for serum PTH in these multivariate analyses did not significantly change the results (data not shown). A sensitivity analysis excluding subjects with diabetes at follow-up (n=30) yielded similar findings for the above associations (data not shown). In addition, no significant interaction between 25(OH)D and sex, ethnicity, BMI or season was found in any of the regression models (p≥0.07).

Baseline serum 25(OH)D according to glycemic progression status is described in Figure 7-1. Those who remained NGT (n=352) or regressed to NGT (n=6) at follow-up had significantly higher serum 25(OH)D levels compared to those who were dysglycemic at follow-up (n=131) (59.84 ± 23.07 nmol/L vs. 53.03 ± 23.16 nmol/L respectively, p=0.0041). Multivariate logistic regression analyses indicated a significant reduced risk of progression to dysglycemia per SD increase in baseline serum 25(OH)D after adjustment for age, sex, ethnicity, season, and baseline and change in both physical activity and vitamin D supplement use (Figure 7-2). However, this association was attenuated with additional adjustment for baseline and change in BMI (OR 0.78, 95% CI 0.59-1.02). In addition, results were essentially the same in sensitivity analyses additionally adjusting for PTH or for family history of type 2 diabetes and baseline C-reactive protein.

7.5 Discussion

The current study found that baseline vitamin D status was an independent predictor of better β-cell function and AUC\textsubscript{glucose} after 3 years of follow-up in the PROMISE study cohort. This is the first study to examine the prospective association of serum 25(OH)D with β-cell function.

Previous studies assessing the association between 25(OH)D and β-cell function have used cross-sectional designs, and have reported inconsistent findings (2,24,29-32,35,345). These inconsistencies may be attributed to the use of less-detailed fasting-based surrogate measures of β-cell function (e.g. HOMA-β or C-peptide) in the majority of previous studies (2,29,30,32,35). Only two studies have used gold-standard methods, including the hyperglycemic clamp (24) and the IVGTT (31), but no significant association between
25(OH)D and β-cell function was found after adjusting for potential confounders. In contrast, the findings in the current study support our cross-sectional results in the PROMISE cohort (345), in which OGTT-based measures of β-cell function, specifically IGI/IR and ISSI-2, were significantly associated with 25(OH)D. Other cross-sectional studies have also reported a similar significant association of 25(OH)D with β-cell function (29,30). In addition to the observational literature, a limited number of intervention studies have examined the effect of vitamin D supplementation on measures of β-cell function (29,35,268,270,271,274,278,279); these studies have similarly yielded inconsistent results. However, most studies included small sample sizes, a short duration of intervention, variation in vitamin D doses, and surrogate measures of β-cell function including C-peptide and HOMA-β, both of which on their own do not account for background IR. Three previous intervention studies (274,278,279) used gold standard IVGTT-based measures of insulin secretion, with one study reporting that supplementation of 1,332 IU vitamin D$_3$ per day resulted in increased first-phase insulin secretion (274). However, given that this study was not a randomized controlled trial, that it included only 10 study participants, and that the IVGTTs were not performed according to standard procedures, their finding should be interpreted with caution. The remaining two studies using IVGTT (278,279) found no effect of supplementation with a synthetic analogue of the active vitamin D metabolite, calcitriol [i.e. 1,25(OH)$_2$D], on insulin secretion in subjects with IGT. It is clear that current evidence is limited and inconsistent regarding the association of 25(OH)D with β-cell function.

In contrast to the findings regarding β-cell function, the current study did not find a significant association of baseline 25(OH)D with follow-up measures of IR. Most cross-sectional studies have found significant inverse associations between 25(OH)D and IR (2,23-26,339,357,358), including our recent study in the PROMISE cohort (345). However, some studies have reported no association (23,31,32,359). In addition, only two prospective studies have been conducted to date (11,58). Forouhi et al. (58) reported a significant inverse association of baseline serum 25(OH)D with HOMA-IR after 10 years of follow-up in Caucasian subjects from the U.K. More recently, Gagnon et al. (11) found a significant positive association of baseline 25(OH)D with insulin sensitivity (HOMA-S) at 5 years in adults participating in the Australian Diabetes, Obesity and Lifestyle Study. Likewise, we also report an initial significant inverse association of baseline 25(OH)D with follow-up
HOMA-IR in the current study, which was attenuated to nonsignificance after adjustment for obesity. Given that our study population is more obese than the populations in these previous studies, it is possible that obesity was a stronger determinant of IR than 25(OH)D in this population.

The current study also found a significant inverse association of baseline serum 25(OH)D with AUC\textsubscript{glucose} at follow-up, indicating that those with higher baseline 25(OH)D had significantly better glucose homeostasis during the follow-up OGTT, even after adjusting for baseline AUC\textsubscript{glucose}. Most previous studies have reported significant inverse associations of 25(OH)D with various continuous measures of glycemia, including fasting or 2-h glucose during the OGTT (2,23,24,28,359). Forouhi et al. (58) also reported a significant inverse association of baseline 25(OH)D with 2-h OGTT glucose, but not fasting glucose, after 10 years of follow-up, with multivariate adjustment.

In addition to the continuous outcome measures assessed, this study also examined the association between baseline serum 25(OH)D and risk of progression to dysglycemia at follow-up. The initial multivariate logistic regression analyses indicated a significant reduced risk of progression to dysglycemia with greater baseline 25(OH)D, but this association was attenuated to nonsignificance with additional adjustment for BMI in model 3. Although there is limited evidence, most previous studies have found an inverse association between vitamin D and diabetes risk (7-10,360,361), but negative findings have also been reported (17,18,20). As was documented in the current study, some investigators have also reported attenuation of an initial significant association after BMI adjustment (17,18), but most previous studies have reported a significant association between 25(OH)D and diabetes even after accounting for body composition (8-11,360,361). Vitamin D is a fat-soluble vitamin, and the consistently observed inverse association between 25(OH)D and adiposity is thought to be largely a result of the sequestering of 25(OH)D in adipose tissue, where it is no longer bioavailable (192).

PROMISE study participants are primarily overweight or obese with 72.8% having a BMI \geq 27 kg/m\textsuperscript{2}, and thus the sequestering effect of adipose tissue on vitamin D bioavailability is one potential explanation for the nonsignificant association with dysglycemia, after BMI adjustment in this cohort. However, given that the current study did find a significant prospective association of baseline 25(OH)D with \(\beta\)-cell function and continuously measured
glycemia, increased power provided through a longer follow-up duration may be needed to detect a significant association of 25(OH)D with risk of progression to dysglycemia.

The current study has a number of potential limitations. First, only baseline 25(OH)D was collected, and an additional serum 25(OH)D measurement at follow-up to examine the effect of longitudinal changes in 25(OH)D on the outcome measures would have strengthened the study. Second, no information on diet was collected, but we did have information on participants’ vitamin D supplement use, which is an important contributor to 25(OH)D levels. In addition, gold standard measures of IR and β-cell function were not used because these procedures are costly and invasive and therefore not feasible for large epidemiological studies. However, the current study used extensively validated proxy measures to determine IR and β-cell dysfunction based on glucose and insulin values from multiple time points in the OGTT. Further, we did not have glucose data for 60 and 90 min during the OGTT, which would have allowed for increased accuracy in the calculation of AUC_{glucose}. It is also important to note possible bias in our results given that those who returned for the follow-up clinic visit were more likely to be older, female and Caucasian than those who did not return. However, we did adjust for these variables in our multivariate analyses. Lastly, because this was an observational study residual confounding is possible because unmeasured confounders may impact the association of serum 25(OH)D with the outcomes. Strengths of this study include its prospective design which allows for the temporality of the associations to be observed. In addition, the current study examined a multiethnic cohort whereas most previous studies have focused solely on Caucasian populations. Examining non-Caucasian ethnic populations is valuable considering these individuals are at high risk for type 2 diabetes and are known to have low 25(OH)D concentrations. The current study also included the direct measurement of serum 25(OH)D, versus reliance on diet and sun-exposure data. In addition, multivariate analyses were adjusted for numerous potential confounders including vitamin D supplement use, which has been excluded in most previous studies (2,24,32,58).

In conclusion, the current study found that higher baseline 25(OH)D independently predicted better β-cell function and lower AUC_{glucose} after 3 years of follow-up, even after adjustment for baseline β-cell function and AUC_{glucose}, respectively. Higher 25(OH)D levels also were
associated with a reduced risk of progressing to dsglycemia, although this association was not statistically significant after adjustment for obesity (adjusted odds ratio 0.78 [95% CI 0.59-1.02]). Longer follow-up of this cohort may reveal a significant inverse association of 25(OH)D with risk of type 2 diabetes. These results support a potential role for vitamin D in the etiology of type 2 diabetes.
Table 7-1. Participant characteristics at baseline and at 3-year follow-up, n=489

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>Δ</th>
<th>% change</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>58.01 ± 23.26</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>PTH (pmol/L)</td>
<td>4.55 ± 1.68</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Anthropometry</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.75 ± 19.70</td>
<td>86.27 ± 19.67</td>
<td>0.9 (−2.15, 4.20)</td>
<td>1.06%</td>
<td>0.0003</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.33 (26.72, 4.57)</td>
<td>30.43 (26.93, 4.58)</td>
<td>0.39 (−0.70, 1.56)</td>
<td>1.23%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>98.43 ± 15.43</td>
<td>99.12 ± 15.60</td>
<td>0.50 (−3.00, 4.70)</td>
<td>0.93%</td>
<td>0.0199</td>
</tr>
<tr>
<td>Physical activity (MET-h/wk)</td>
<td>19.59 (7.39, 53.52)</td>
<td>23.13 (9.62, 59.66)</td>
<td>1.56 (−10.92, 15.29)</td>
<td>3.94%</td>
<td>0.1087</td>
</tr>
<tr>
<td>Smoking (%current)</td>
<td>30 (6.29)</td>
<td>25 (5.13)</td>
<td>−5 (1.16)</td>
<td>−16.67%</td>
<td>0.09</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>125.92 ± 16.03</td>
<td>125.91 ± 15.02</td>
<td>0.00 (−7.5, 9.5)</td>
<td>0.80%</td>
<td>0.79</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>80.16 ± 10.32</td>
<td>80.17 ± 10.10</td>
<td>1.00 (−5.5, 6.5)</td>
<td>0.86%</td>
<td>0.76</td>
</tr>
<tr>
<td>Vitamin D Supplement Use, n (%)</td>
<td>212 (43.35)</td>
<td>262 (53.58)</td>
<td>50 (10.23)</td>
<td>24.04%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>4.95 ± 0.53</td>
<td>5.20 (4.8, 5.6)</td>
<td>0.30 (0.0, 0.6)</td>
<td>6.90%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2-hour glucose (mmol/L)</td>
<td>5.72 ± 1.37</td>
<td>6.10 (5.1, 7.6)</td>
<td>0.65 (−0.45, 1.80)</td>
<td>11.87%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin Sensitivity</td>
<td>ISOGTT index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISOGTT index</td>
<td>13.45 (8.52, 20.79)</td>
<td>11.54 (6.89, 18.85)</td>
<td>−1.65 (−5.64, 1.62)</td>
<td>−16.13%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin Resistance</td>
<td>HOMA-IR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.88 (1.19, 3.09)</td>
<td>2.27 (1.41, 3.76)</td>
<td>0.34 (−0.25, 1.00)</td>
<td>21.42%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Beta-cell Function</td>
<td>IGI/IR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGI/IR</td>
<td>9.55 (5.43, 14.94)</td>
<td>7.41 (4.49, 13.70)</td>
<td>−1.30 (−4.62, 1.53)</td>
<td>−20.80%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ISSI-2</td>
<td>727.49 (568.74, 907.48)</td>
<td>613.51 (493.85, 823.69)</td>
<td>−93.31 (−219.93, 27.72)</td>
<td>−14.44%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AUCglucose</td>
<td>13.77 ± 2.29</td>
<td>14.82 ± 3.24</td>
<td>0.83 (−0.5, 2.5)</td>
<td>5.76%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are n (%) for categorical variables, mean ± SD for continuous variables, or median (25% & 75% interquartiles) for non-normally distributed variables. Data are for all participants at follow-up with a baseline serum 25(OH)D measurement (n=489). Tests of significance are the McNemar’s test for categorical variables, the paired Student t test for normally-distributed variables and Wilcoxon signed-rank test for non-normally distributed variables. Percentage change calculated as [((follow-up – baseline)/baseline)*100].
Table 7-2. Multiple linear regression analysis of associations of baseline 25(OH)D (nmol/L) with measures of insulin sensitivity/resistance and β-cell function and AUCglucose at the 3-year follow-up

<table>
<thead>
<tr>
<th>Outcome per unit increase in baseline 25(OH)D</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>P value</td>
<td>R²</td>
</tr>
<tr>
<td>IS-OGTT *</td>
<td>0.002 (-0.0004, 0.004)</td>
<td>0.12</td>
<td>0.49</td>
</tr>
<tr>
<td>HOMA-IR *</td>
<td>-0.003 (-0.005, -0.0006)</td>
<td>0.014</td>
<td>0.36</td>
</tr>
<tr>
<td>IGI/IR *</td>
<td>0.007 (0.003, 0.010)</td>
<td>0.0003</td>
<td>0.18</td>
</tr>
<tr>
<td>ISSI-2 *</td>
<td>0.002 (0.0004, 0.003)</td>
<td>0.014</td>
<td>0.36</td>
</tr>
<tr>
<td>AUCglucose *</td>
<td>-0.001 (-0.002, -0.0005)</td>
<td>0.0010</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Model 1: Adjusted for age, sex, ethnicity, season of 25D measurement and baseline outcome variable
Model 2: Adjusted as in model 1 plus baseline physical activity, change in physical activity, baseline vitamin D supplement use, change in vitamin D supplement use
Model 3: Adjusted as in model 2 plus baseline BMI and change in BMI
* Log transformations
Figure 7-1. Baseline serum 25(OH)D by glycemic progression status at follow-up.

NGT, normal glucose tolerance; Pre-DM refers to impaired fasting glucose (IFG) or impaired glucose tolerance (IGT); DM, type 2 diabetes.
Note: Based on ANOVA, none of the pairwise associations were statistically significant.
Figure 7-2. Multivariate logistic regression analysis of associations of baseline 25(OH)D with progression to dysglycemia at follow-up

Model 1: Adjusted for age, sex, season, and ethnicity
Model 2: Adjusted as in Model 1 plus baseline physical activity, change in physical activity, baseline vitamin D supplement use, and change in vitamin D supplement use
Model 3: Adjusted as in Model 2 plus baseline BMI and change in BMI

Progression to dysglycemia defined as having developed impaired fasting glucose, impaired glucose tolerance or incident type 2 diabetes based on the oral glucose tolerance test at the three-year follow-up.
CHAPTER 8:

EFFECT OF SEASONAL CHANGES IN 25(OH)D ON INSULIN RESISTANCE AND BETA-CELL DYSFUNCTION OVER TIME: A PILOT STUDY
Chapter 8: Effect of Seasonal Changes in 25(OH)D on Insulin Resistance and Beta-Cell Dysfunction Over Time: A Pilot Study

8.1 Abstract

**Background:** Seasonal variation in 25(OH)D levels is well described, with levels being highest in the summer and lowest in the winter. It has been postulated that seasonal fluctuations in 25(OH)D may have adverse effects, and that these effects may be tissue-specific. The objective of this study was to assess how seasonal variation in serum 25(OH)D would influence measures of insulin resistance (IR) and beta (β)-cell function over time.

**Research Design and Methods:** We followed 127 individuals, age 53 ± 10y, for approximately 2.5 years. Participants completed two clinic visits approximately 2.5 years apart, and which were seasonally discordant (season change group: summer→winter or winter→summer). At both clinic visits, serum 25(OH)D was measured and 75g oral glucose tolerance tests (OGTT) were administered. Insulin resistance was measured using the Matsuda index (IS_{OGTT}) and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR); and β-cell function was determined using both the insulinogenic index divided by HOMA-IR (IGI/IR) and the Insulin Secretion Sensitivity Index-2 (ISSI-2). Linear mixed-effects models were used to assess the effect of seasonal changes in 25(OH)D on measures of insulin resistance and β-cell function over time, accounting for repeated measures in the same individual, and adjusting for time, supplement use, physical activity, and BMI.

**Results:** Serum 25(OH)D concentration at the first clinic visit was 74.94 ± 26.68 nmol/L, and increased overall in both season change groups (summer→winter, winter→summer). However, the increase in 25(OH)D in the winter→summer group was significantly greater than that in the summer→winter group. Measures of insulin resistance and β-cell function decreased overall in both season change groups. There was no significant independent interaction effect of season change group and 25(OH)D on insulin resistance and β-cell function over time. Rather, time was a significant determinant of these outcomes in all of the multivariate models.
Conclusions: Seasonal changes in 25(OH)D do not appear to have an effect on insulin resistance and β-cell function over time. Additional studies in a larger cohort and with repeat measurements in consecutive seasons are warranted to confirm this study’s findings.
8.2 Introduction

Emerging evidence suggests a potential role for serum 25-hydroxyvitamin D (25(OH)D) in insulin resistance (IR) and beta (β)-cell dysfunction, the main underlying pathophysiological disorders of type 2 diabetes (T2DM). Several prospective studies have reported a significant inverse association of serum 25(OH)D with diabetes risk (7-9,11,12), but results have not been consistent (17-19). In addition, there have been a limited number of studies investigating the prospective relationship of 25(OH)D with insulin resistance and β-cell function (11,58,362). These studies have all relied on a single baseline 25(OH)D measurement and therefore may not adequately reflect changes in 25(OH)D over time. Given the seasonal variation in 25(OH)D levels (202,203,324,363-365), it has been postulated that such seasonal fluctuations may have adverse effects (i.e. increase risk of certain conditions) and that these effects may be tissue specific (59). Previous studies have reported seasonal variation in diabetes risk, with poorer glycemic control and greater diabetes incidence during the winter compared to the summer (244), which may be attributable to seasonal changes in serum 25(OH)D. However, the effects of the seasonal variation in 25(OH)D levels on insulin resistance and β-cell function over time are currently unknown. Therefore, the objective of the current study was to examine how seasonal changes in 25(OH)D relate to changes in insulin resistance and β-cell function over time in the same individual.

8.3 Methods

8.3.1 Study Design

Details regarding the PROspective Metabolism and ISlet cell Evaluation (PROMISE) cohort study have been published previously (328,362). PROMISE participants, who were aged 30 years and older at baseline, were recruited from Toronto (latitude 43° 40' N) and London (latitude 43° 02' N), Ontario, Canada, between May 2004 and December 2006. Participants were at high risk for type 2 diabetes as they were recruited based on the presence of one or more risk factors for diabetes including obesity, hypertension, a family history of diabetes and/or a history of gestational diabetes or birth of a macrosomic infant (328). In addition to
clinic visits every three years, participants were contacted annually after the baseline visit to update contact information and collect data on major health events.

A subset of the PROMISE cohort was selected for the current study, with their follow-up visit arranged to allow for seasonal discordance between the 3-year and 6-year clinic visits. Specifically, those with a 3-year clinic visit between May 2007 and April 2008 were asked to return for their 6-year clinic visit in the opposite season of their previous visit. The time frame selected for the 3-year visits for inclusion in this substudy represented the beginning of the first follow-up clinic visit for PROMISE participants and was chosen to minimize additional confounding factors with 3-year clinic visits spanning over multiple years.

Season was defined using the date participants completed each clinic visit and was categorized as follows: May-October (summer/early fall); November-April (winter/early spring). This categorization of seasons was determined based on previous findings of serum 25(OH)D levels in a population residing in Toronto, Canada (203). In the present study, individuals who had a 3-year clinic visit in the summer (May-Oct 2007) were invited for an earlier 6-year follow-up visit in the winter (Jan-Apr 2010). Similarly, those with a 3-year visit in the winter (Nov 2007-Apr 2008) were invited for an earlier 6-year follow-up visit in the summer (May-Oct 2010). For purposes of clarity, the 3-year visit and 6-year visit will now be referred to as visit 1 and visit 2, respectively. Season change group refers to the two seasonally discordant groups, specifically summer→winter versus winter→summer (see Table 8-1).

8.3.2 Measures

At both clinic assessments, fasting blood samples were collected and 75-gram oral glucose tolerance tests (OGTTs) were conducted with additional blood samples collected at 30- and 120- minutes for glucose and insulin measurements. Further details regarding the measurements of glucose and insulin can be found elsewhere (Chapter 4).

Serum 25(OH)D levels were measured at both clinic visits using the DiaSorin “25 OH Vitamin D TOTAL” competitive chemiluminescent immunoassay on the automated LIAISON® analyzer (Stillwater, MN). This assay has 100% specificity for both 25(OH)D$_2$
and 25(OH)D$_3$. The detection limit of the assay is 10 nmol/L, and we found that it has an intra-assay coefficient of variation (CV) of 6.7% and an inter-assay CV of 11.6%. The 25(OH)D TOTAL method has been validated against the DiaSorin radioimmunoassay (RIA) ($r=0.92$), which is the first test approved for clinical diagnosis by the Food and Drug Administration and which is also the most widely used method (219). Parathyroid hormone (PTH) was also measured in blood samples from both clinic visits using an electrochemiluminescence immunoassay on the Roche Modular E170 analyzer (Laval, QC), which has a detection range from 0.127-530 pmol/L.

Anthropometric measurements as well as assessment of additional potential covariates were examined at both clinic visits. Details regarding the measurement of anthropometric variables, blood pressure, and physical activity have been previously reported (Chapter 6). Supplement use, specifically any vitamin or multivitamin containing vitamin D, was obtained through an open-ended question on current medication use. Ethnicity, smoking and the participant’s family history of diabetes were assessed using structured questionnaires.

Measures of insulin resistance and β-cell function were assessed at both clinic visits. Insulin resistance was quantified using both the IS$_{OGTT}$ index of Matsuda and Defronzo (134) and the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index of Matthews and colleagues (132). The IS$_{OGTT}$ index, which is a measure of insulin sensitivity (134), is defined as $10000 / \sqrt{(\text{FPG} \times \text{FPI}) \times (\text{G} \times \text{I})}$, where FPG = fasting plasma glucose, FPI = fasting plasma insulin, G = mean glucose during the oral glucose tolerance test, and I = mean insulin during the oral glucose tolerance test. This index reflects whole body insulin sensitivity, and has been validated against the euglycemic-hyperinsulinemic clamp technique (134). HOMA-IR, which is defined as $\text{FPG} \times \text{FPI} / 22.5$ (132), largely reflects hepatic insulin resistance, and has also been validated against the clamp (132). β-cell function was calculated by taking the insulinogenic index divided by HOMA-IR (IGI/IR) (144), which is a widely used measure of β-cell function. The insulinogenic index is calculated by taking the ratio of (30 min insulin - fasting insulin) to (30 min glucose - fasting glucose) (144), and has been validated against gold standard measures of insulin secretion (1st phase insulin secretion on intravenous glucose tolerance test). The Insulin Secretion Sensitivity Index 2 (ISSI-2), which is a more recently proposed measure of β-cell function that is analogous to the disposition index but
derived from the oral glucose tolerance test (149), was also calculated. This index, which has been validated against directly measured disposition index (150), is defined as the ratio of the area-under-the-insulin curve (AUC_{insulin}) to the area-under-the-glucose curve (AUC_{glucose}), multiplied by IS_{OGTT} (150). Glucose tolerance status was assessed using the 1999 World Health Organization criteria (341). In addition, participants who reported being diagnosed with type 2 diabetes during the follow-up period through either the annual telephone contacts or the clinic examinations were also considered as having incident diabetes at follow-up. Verification of self-reported diabetes was obtained from the participant’s physician through a supplementary form requesting information on date of diagnosis, blood glucose levels supporting the diagnosis, and current treatment.

8.3.3 Statistical Analysis

SAS version 9.2 was used for all analyses. Continuous variables were reported as mean ± SD or median with interquartile ranges in the case of skewed distributions, while categorical variables were reported as n (%). Natural logarithmic transformations were applied for all non-normally distributed variables. Changes in participant characteristics between the two clinic visits in the entire study population and stratified by season change group were tested using the McNemar test for categorical variables and paired Student t test or Wilcoxon signed-rank test for normally-distributed or non-normally distributed continuous variables, respectively. Percentage change was calculated as follow-up value minus baseline value divided by the baseline value, multiplied by 100.

Linear mixed-effects models were used to analyze the effects of seasonal changes in 25(OH)D on log-transformed measures of insulin resistance and β-cell function over time. Such models are widely used to analyze longitudinal data as they account for the lack of independence between repeated measurements in the same participants (366,367). The dependent variables for the mixed models analyses were the log-transformed measures of insulin resistance or β-cell function at both clinic visits. The primary exposure variable was the main interaction effect of serum 25(OH)D x season change group. Separate models were used for each outcome variable, and all models included adjustment for visit number (as a
covariate of time), supplement use, physical activity and BMI. The models used a compound symmetry covariance structure given only two repeated measurements on each participant.

8.4 Results

One hundred and sixty three PROMISE participants were eligible to be included in the current substudy based on the dates of their 3-year clinic visit, as described above. Of these 163 participants, 30 were classified as diabetic and therefore excluded. An additional two participants did not have seasonal discordance between visit 1 and visit 2, and four participants had missing 25(OH)D measurements. Therefore, for the purposes of this study, these participants were excluded. Overall, 127 participants were included for examination in the current study. There were 43 participants who belonged to the season change group summer→winter; 84 participants belonged to the winter→summer season change group.

Participant characteristics at the two clinic visits, as well as percent change in these participant characteristics over the follow-up period are presented in Table 8-2. At visit 1, the mean serum 25(OH)D concentration was 74.94 ± 26.68 nmol/L, where approximately 16% had 25(OH)D levels less than 50 nmol/L, and 49% had 25(OH)D levels less than 75 nmol/L. Overall, there were significant increases in 25(OH)D levels and vitamin D supplement use over the follow-up period. In addition, PROMISE participants had worsening glucose homeostasis during follow-up, reflected by a significant decrease in ISOGTT and a significant increase in HOMA-IR, as well as significant decreases in both measures of β-cell function, specifically IGI/IR and ISSI-2. Further, regarding glucose tolerance status at visit 1, 21 participants (16.5%) had impaired fasting glucose or impaired glucose tolerance, with the majority of participants (n=106, 83.5%) having normal glucose tolerance. By visit 2, although most participants had normal glucose tolerance (n=94, 74%), 1 person had developed diabetes, 23 (18.1%) participants had developed impaired fasting glucose or impaired glucose tolerance, and the remaining 103 (81.1%) had maintained their glycemic status.

Changes in participant characteristics, specifically 25(OH)D and the primary outcome measures, over the follow-up period stratified by the seasonal change groups are shown in Table 8-3 (summer→winter) and Table 8-4 (winter→summer). Serum 25(OH)D increased
in both seasonal change groups; however the increase in 25(OH)D in the participants of the summer→winter group was not significant (Table 8-3). In addition, there was a significantly greater increase in 25(OH)D levels in the winter→summer group compared to those in the summer→winter group (Table 8-5). Further, as is evident from Figures 8-1a and 8-1b, there was significant between-individual variation in change in serum 25(OH)D over the two clinic visits. Changes in other participant characteristics including BMI and physical activity did not significantly differ between the two seasonal change groups. Measures of insulin resistance and β-cell function worsened over time and in both seasonal change groups (Tables 8-3 and 8-4), with greater declines in the summer→winter group for all measures except ISOGTT. However, there were no significant differences in the changes of these measures over time between the two seasonal change groups (Table 8-5).

Results from the mixed models analysis testing the effect of seasonal changes in 25(OH)D on insulin resistance and β-cell function are shown in Tables 8-6a to 8-6d. In Tables 8-6a and 8-6b, it is evident that the interaction effect of 25(OH)D with season change group on both measures of insulin resistance, specifically ISOGTT and HOMA-IR, was not significant. Serum 25(OH)D itself was, however, a significant determinant of both measures of insulin resistance over time, in the minimally-adjusted and fully-adjusted models. Similarly, in Tables 8-6c and 8-6d, the interaction effect of 25(OH)D with season change group on both measures of β-cell function, IGI/IR and ISSI-2, was not significant. Although 25(OH)D was a significant determinant of both IGI/IR and ISSI-2 over time in the minimally adjusted model, this association was attenuated to non-significance with further multivariate adjustment, particularly after adjustment for BMI. Time, however, was a significant determinant of all measures of insulin resistance and β-cell function over time, highlighting the progressive nature of these disorders in these high-risk subjects. Sensitivity analyses were also conducted to assess the effect of additional adjustment for ethnicity in the mixed models analyses; however findings were essentially the same. In addition, stratifying by ethnicity (Caucasian vs. non-Caucasian) did not significantly change study findings (data not shown).

Given that the expected change in 25(OH)D between season change groups was not observed, a regression analysis regardless of season change group, indicated an initial significant inverse association of change in 25(OH)D with change in insulin resistance, but
no significant association of change in 25(OH)D with change in β-cell function over time (data not shown). However, all associations were attenuated to non-significance after further multivariate adjustment.

8.5 Discussion

The present study found no significant effect of seasonal changes on 25(OH)D or on measures of insulin resistance and β-cell function over time. Rather, time was a significant determinant of changes in these measures of glucose homeostasis over the follow-up period. To our knowledge, this is the first study to examine the potential effect of seasonal fluctuations in 25(OH)D on the underlying disorders of type 2 diabetes.

Previous research has reported marked seasonal variation in serum 25(OH)D in those at high latitudes (202,203,324,363-365). This is a result of the lack of endogenous vitamin D synthesis during the winter months. It has been previously demonstrated that at latitudes of 41°N (Boston, USA), the “vitamin D winter” extends from November to early March where there is insufficient UVB radiation available for cutaneous vitamin D synthesis (185). Further, at latitudes farther north, like Edmonton, Canada (52°N), the “vitamin D winter” extends from mid-October to mid-March (185). Therefore, and as has been previously reported in several Canadian studies, serum 25(OH)D levels are generally higher during the summer months and lower in the winter months (202,203,324,364,365,368). In the current study, 25(OH)D increased in the winter→summer group, as expected. Surprisingly, however, we also found that 25(OH)D levels increased in the summer→winter group. Despite the increase in 25(OH)D in both seasonal change groups, those in the winter→summer group had a significantly larger increase in 25(OH)D than those in the summer→winter group. Although the increase in 25(OH)D in the summer→winter group could be due to increases in vitamin D supplement use, only 3 participants in this group started vitamin D supplementation during the follow-up period. It is also important to keep in mind that the clinic visits were separated by approximately 2.5 years and therefore the changes in 25(OH)D may not adequately reflect seasonal fluctuations from consecutive seasons. Further analysis into individual change in 25(OH)D over the follow-up period demonstrated significant inter-individual variation, with some participants increasing, decreasing, or
maintaining their 25(OH)D levels in both seasonal-change groups. This significant individual variation in changes in 25(OH)D levels has been previously reported (369,370) and can be attributed to a variety of factors including age, ethnicity, sun-exposure, diet, as well as genetics (153,370,371).

Despite the overall increases in 25(OH)D during the follow-up period, insulin resistance and β-cell function worsened in all participants which was expected as they are at risk for type 2 diabetes. However, no significant differences in these changes in insulin resistance and β-cell function between the two seasonal change groups were observed. Although previous prospective studies have reported a significant inverse association of 25(OH)D with insulin resistance (11,58) and as shown within the larger PROMISE cohort, a significant positive association of 25(OH)D with β-cell function (362), these studies did not assess the effect of change in 25(OH)D, or specifically seasonal change in 25(OH)D, on the outcome measures. Previous studies have reported on the seasonal variation in diabetes incidence and glycemic control (244,372-375). Yet, limited and inconsistent findings have been reported on the seasonality of insulin resistance specifically (376-378). Although vitamin D has been postulated as a potential explanation for these findings on the seasonality of diabetes risk, the current substudy was not designed or powered to investigate this association specifically. Rather, no study has yet investigated the longitudinal effect of seasonal changes in 25(OH)D on diabetes risk. The findings in the current study suggest that seasonal changes in 25(OH)D are not an important determinant of insulin resistance and β-cell function over time. Rather, time itself was found to be a notable determinant in this PROMISE cohort, and is evident of their progression to dysglycemia given their high-risk metabolic state.

Recently, Vieth (59) proposed that a greater seasonal amplitude of 25(OH)D observed in those at higher latitude may be associated with increased risk of certain chronic diseases, specifically prostate and pancreatic cancers. The potential harmful effects of the seasonal fluctuations in serum 25(OH)D may be tissue-specific, however, as the adaptation of non-renal tissues to modest changes in serum 25(OH)D has never been characterized. In the current study however, the expected seasonal drop in 25(OH)D from summer→winter was not observed. Yet given that the winter→summer group had a significant and greater increase in 25(OH)D than the summer→winter group, a-priori analyses were conducted to assess if
there would be any different effect on the outcome measures between the two season change groups. The results from the mixed models analyses in the current study indicate that the changes in 25(OH)D between the two season change groups (winter→summer vs. summer→winter) did not have a significant effect on changes in insulin resistance and β-cell function. However, and as previously mentioned, given that the expected seasonal drop in serum 25(OH)D from summer to winter was not observed, it is difficult to truly draw conclusions on the effect of expected seasonal fluctuations in 25(OH)D over time on measures of insulin resistance and β-cell function. Additional studies are needed with measurements of 25(OH)D, insulin resistance and β-cell function in consecutive seasons to further investigate whether seasonal fluctuations in serum 25(OH)D have an adverse effect on these measures of glucose homeostasis.

Strengths of this study include its novel design of seasonally discordant clinic visits (winter→summer versus summer→winter), as well as multiple measurements of serum 25(OH)D in the same participants over time. Specifically in the context of type 2 diabetes, this is the first study to our knowledge which has examined longitudinal changes in 25(OH)D on insulin resistance and β-cell function using more than one 25(OH)D measurement. Only one previous study has been conducted using repeat measurements of 25(OH)D over a mean follow-up of 2.7 years (13). Although this study found that higher 25(OH)D (measured at baseline, 6 months and then annually up until 4 years) was significantly associated with a lower risk of developing diabetes, measures of insulin resistance and β-cell function specifically were not assessed. In addition, the use of mixed models in the current study was beneficial as this statistical approach offers flexibility in longitudinal studies and accounts for the lack of independence between the repeated measurements at the two clinic visits from the same participants. This study, however, has a number of limitations. First, the sample size was somewhat small and therefore insufficient power may have reduced the ability to detect significant effects. Second, because this substudy was designed within the PROMISE cohort, attempts were made to maintain its original design and therefore approximately 2.5 years separated the seasonally-discordant clinic visits. Therefore, given that repeat measurements were not made in consecutive seasons, this may have influenced our ability to observe true seasonal fluctuations in serum 25(OH)D that occur within the same year. Further, it is possible that we would have seen a significant difference if we had sampled only during the
peak (end of summer) and nadir (end of winter) time points for seasonal changes in serum 25(OH)D status, rather than throughout the summer and winter seasons. Lastly, information on vitamin D dietary intake and participant’s sun exposure behaviour were only assessed at the 6-year clinic visit and therefore we could not examine how changes in these factors over the follow-up period contributed to changes in 25(OH)D and our primary outcome measures.

In conclusion, the present study found no significant effect of seasonal changes in 25(OH)D on insulin resistance and β-cell function in a cohort of subjects followed for approximately 2.5 years. Rather, time appeared to be a significant determinant of changes in baseline 25(OH)D and the underlying disorders of type 2 diabetes. Additional studies are needed to elucidate whether seasonal fluctuations in 25(OH)D have an adverse effect on insulin resistance and β-cell function with a larger cohort and with measurements from consecutive seasons in the same participants.
## Table 8-1. Design of Substudy

<table>
<thead>
<tr>
<th>Season Change Group</th>
<th>Summer→Winter</th>
<th>Winter→Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>Winter</td>
<td>Winter</td>
</tr>
<tr>
<td>Visit 1 (3-year visit)</td>
<td>May – Oct 07</td>
<td>Nov 07 – Apr 08</td>
</tr>
<tr>
<td>Visit 2 (6-year visit)</td>
<td>Jan – Apr 2010</td>
<td>May – Oct 2010</td>
</tr>
</tbody>
</table>
Table 8-2. Association of participant characteristics by 3-year (visit 1) and 6-year (visit 2) follow-up visits, n=127

<table>
<thead>
<tr>
<th>Variable</th>
<th>Visit 1 (3-year follow-up)</th>
<th>Visit 2 (6-year follow-up)</th>
<th>median Δ</th>
<th>% change</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (%F)</td>
<td>88 (69.29)</td>
<td>88 (69.29)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>90 (70.87)</td>
<td>90 (70.87)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>52.80 ± 9.50</td>
<td>55.28 ± 9.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>74.94 ± 26.68</td>
<td>82.28 ± 28.12</td>
<td>7.0 (-5, 18)</td>
<td>10%</td>
<td>0.0002</td>
</tr>
<tr>
<td>PTH (pmol/L)</td>
<td>4.60 (3.8, 6.3)</td>
<td>4.3 (3.3, 5.6)</td>
<td>-0.30 (-1.4, 0.4)</td>
<td>-5.19%</td>
<td>0.0004</td>
</tr>
<tr>
<td>Anthropometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.95 (25.85, 32.71)</td>
<td>28.98 (25.47, 33.05)</td>
<td>-0.10 (-1.17, 1.10)</td>
<td>-0.43%</td>
<td>0.77</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>93.95 (84.85, 104.55)</td>
<td>96.45 (88.25, 106.65)</td>
<td>2.00 (-0.70, 6.95)</td>
<td>2.17%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Physical activity (MET-h/wk)</td>
<td>23.95 (11.29, 54.62)</td>
<td>25.91 (13.15, 55.24)</td>
<td>0.86 (-12.29, 11.12)</td>
<td>1.84%</td>
<td>0.83</td>
</tr>
<tr>
<td>Vitamin D Supplement Use, n (%)</td>
<td>72 (56.69)</td>
<td>86 (67.72)</td>
<td>14 (11.03)</td>
<td>19.44%</td>
<td>0.0005</td>
</tr>
<tr>
<td>Insulin Sensitivity</td>
<td>ISOGTT index</td>
<td>ISOGTT index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin Resistance</td>
<td>HOMA-IR</td>
<td>HOMA-IR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-cell Function</td>
<td>IGI/IR</td>
<td>ISSI-2</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Data are for all participants at follow-up with a 3-year and 6-year follow-up serum 25(OH)D measurement (n=127). Data are n (%) for categorical variables, mean ± SD for continuous variables, or median (25% & 75% interquartiles) for non-normally distributed variables. Tests of significance are the McNemar test for categorical variables, paired Student t test for normally-distributed variables and Wilcoxon signed-rank test for non-normally distributed variables. Percentage change calculated as [((follow-up – baseline)/baseline)*100]. Δ = Visit 2 – Visit 1
Table 8-3. Association of participant characteristics by seasonal change between visit 1 and visit 2 (summer→winter, n=43)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Summer (visit 1)</th>
<th>Winter (visit 2)</th>
<th>median Δ</th>
<th>% change</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>78.49 ± 24.86</td>
<td>78.56 ± 27.02</td>
<td>4.0</td>
<td>(18.0, 15.0)</td>
<td>4.76 %</td>
</tr>
<tr>
<td>PTH (pmol/L)</td>
<td>4.5 (3.7, 6.3)</td>
<td>4.3 (3.4, 5.6)</td>
<td>-0.40</td>
<td>(-1.3, 0.7)</td>
<td>-10.00 %</td>
</tr>
<tr>
<td>Anthropometry</td>
<td>BMI (kg/m²)</td>
<td>27.95 (25.54, 33.17)</td>
<td>27.21 (25.29, 33.11)</td>
<td>-0.42</td>
<td>(-1.24, 0.86)</td>
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<tr>
<td></td>
<td>Waist circumference (cm)</td>
<td>93.25 (83.90, 104.15)</td>
<td>94.85 (89.75, 103.80)</td>
<td>2.25</td>
<td>(-0.50, 6.95)</td>
</tr>
<tr>
<td>Physical activity (MET-h/wk)</td>
<td>23.95 (10.18, 72.81)</td>
<td>25.20 (14.05, 65.25)</td>
<td>-1.51</td>
<td>(-15.87, 11.01)</td>
<td>-8.68 %</td>
</tr>
<tr>
<td>Vitamin D Supplement Use, n (%)</td>
<td>27 (62.79)</td>
<td>30 (69.77)</td>
<td>3 (6.98)</td>
<td>11.11 %</td>
<td>0.08</td>
</tr>
<tr>
<td>Insulin Sensitivity</td>
<td>ISOGTT index</td>
<td>14.65 (6.70, 21.53)</td>
<td>11.91 (5.78, 19.05)</td>
<td>-0.16</td>
<td>(-5.44, 2.10)</td>
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<td></td>
<td>HOMA-IR</td>
<td>1.73 (1.01, 3.33)</td>
<td>1.76 (1.25, 4.17)</td>
<td>0.20</td>
<td>(-0.31, 1.45)</td>
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<tr>
<td></td>
<td>IGI/IR</td>
<td>10.55 (4.73, 19.95)</td>
<td>6.85 (4.74, 14.29)</td>
<td>-1.10</td>
<td>(-6.50, 0.82)</td>
</tr>
<tr>
<td></td>
<td>ISSI-2</td>
<td>696.08 (561.76, 977.14)</td>
<td>601.71 (451.97, 778.95)</td>
<td>-115.3</td>
<td>(-217.53, 7.23)</td>
</tr>
</tbody>
</table>

Data are n (%) for categorical variables, mean ± SD for normally distributed continuous variables, or median (25% & 75% interquartiles) for non-normally distributed variables. Tests of significance are the McNemar test for categorical variables, paired Student t test for normally-distributed variables and Wilcoxon signed-rank test for non-normally distributed variables. Percentage change calculated as [(follow-up – baseline)/baseline]*100]. Δ = Visit 2 – Visit 1
Table 8-4. Association of participant characteristics by seasonal change between visit 1 and visit 2 (winter→summer, n=84)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Winter (visit 1)</th>
<th>Summer (visit 2)</th>
<th>median Δ</th>
<th>% change</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>75.0 (55.5, 87.0)</td>
<td>81.5 (63.5, 98.5)</td>
<td>9.50</td>
<td>-2.5, 19.5</td>
<td>15.86 %</td>
</tr>
<tr>
<td>PTH (pmol/L)</td>
<td>4.7 (4.05, 6.45)</td>
<td>4.25 (3.30, 5.50)</td>
<td>-0.30</td>
<td>-1.40, 0.35</td>
<td>-8.01 %</td>
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<tr>
<td>Anthropometry</td>
<td>29.71 ± 5.64</td>
<td>29.80 ± 5.90</td>
<td>0.06</td>
<td>-1.12, 1.27</td>
<td>0.26 %</td>
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<td>Waist circumference (cm)</td>
<td>94.95 (84.85, 105.15)</td>
<td>96.98 (88.15, 106.95)</td>
<td>1.45</td>
<td>-0.85, 6.95</td>
<td>1.67 %</td>
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<tr>
<td>Physical activity (MET-h/wk)</td>
<td>23.68 (13.69, 52.10)</td>
<td>26.0 (13.15, 47.37)</td>
<td>1.28</td>
<td>-8.12, 11.44</td>
<td>6.33 %</td>
</tr>
<tr>
<td>Vitamin D Supplement Use, n (%)</td>
<td>45 (53.57)</td>
<td>56 (66.67)</td>
<td>11 (13.1)</td>
<td>24.44 %</td>
<td>0.0023</td>
</tr>
<tr>
<td>Insulin Sensitivity</td>
<td>ISOGTT index</td>
<td>12.79 (8.15, 24.24)</td>
<td>11.37</td>
<td>8.06, 18.88</td>
<td>-1.09 %</td>
</tr>
<tr>
<td>Insulin Resistance</td>
<td>HOMA-IR</td>
<td>1.99 (1.14, 3.14)</td>
<td>2.26</td>
<td>1.49, 3.24</td>
<td>0.18 (-0.40, 0.79)</td>
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<tr>
<td>Beta-cell Function</td>
<td>IGI/IR</td>
<td>7.84 (5.11, 11.94)</td>
<td>6.86</td>
<td>4.21, 11.17</td>
<td>-0.77 (-3.35, 1.42)</td>
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<tr>
<td></td>
<td>ISSI-2</td>
<td>656.22 (532.59, 850.19)</td>
<td>620.45</td>
<td>461.89, 829.98</td>
<td>-57.18 (-175.83, 81.77)</td>
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</tbody>
</table>

Data are n (%) for categorical variables, mean ± SD for normally distributed continuous variables, or median (25% & 75% interquartiles) for non-normally distributed variables. Tests of significance are the McNemar test for categorical variables, paired Student t test for normally-distributed variables and Wilcoxon signed-rank test for non-normally distributed variables. Percentage change calculated as [((follow-up – baseline)/baseline)*100].

Δ = Visit 2 – Visit 1
Table 8-5. Association of change in participant characteristics by seasonal change between visit 1 and visit 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Summer→Winter</th>
<th>Winter→Summer</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>43</td>
<td>84</td>
<td></td>
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<tr>
<td>Δ Serum 25(OH)D (nmol/L)</td>
<td>4.0 (-18.0, 15.0)</td>
<td>9.5 (-2.5, 19.5)</td>
<td>0.026</td>
</tr>
<tr>
<td>Δ PTH (pmol/L)</td>
<td>-0.40 (-1.3, 0.7)</td>
<td>-0.30 (-1.40, 0.35)</td>
<td>0.31</td>
</tr>
<tr>
<td>Δ Anthropometry BMI (kg/m^2)</td>
<td>-0.42 (-1.24, 0.86)</td>
<td>0.06 (-1.12, 1.27)</td>
<td>0.086</td>
</tr>
<tr>
<td>Δ Waist circumference (cm)</td>
<td>2.25 (-0.50, 6.95)</td>
<td>1.45 (-0.85, 6.95)</td>
<td>0.88</td>
</tr>
<tr>
<td>Δ Physical activity (MET-h/wk)</td>
<td>-2.44 (-17.43, 11.01)</td>
<td>1.28 (-8.12, 11.44)</td>
<td>0.61</td>
</tr>
<tr>
<td>Δ Vitamin D Supplement Use (% started)</td>
<td>3 (12%)</td>
<td>12 (80%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Δ Insulin Sensitivity IS_OGTT index</td>
<td>-0.16 (-5.44, 2.10)</td>
<td>-1.09 (-3.22, 1.45)</td>
<td>0.94</td>
</tr>
<tr>
<td>Δ Insulin Resistance HOMA-IR</td>
<td>0.20 (-0.31, 1.45)</td>
<td>0.18 (-0.40, 0.79)</td>
<td>0.39</td>
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<tr>
<td>Δ Beta-cell Function ISSI-2</td>
<td>-1.10 (-6.50, 0.82)</td>
<td>-0.77 (-3.35, 1.42)</td>
<td>0.83</td>
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<tr>
<td></td>
<td>-115.53 (-217.53, 7.23)</td>
<td>-57.18 (-175.83, 81.77)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Data are for all participants at follow-up with a 3-year and 6-year follow-up serum 25(OH)D measurement (n=127). Data are n (%) for categorical variables or median (25% & 75% interquartiles) for non-normally distributed variables. P values are for the Kolmogorov-Smirnov test for non-parametric distributions between independent groups. The Mantel-Haenszel statistic was used for categorical variables. Δ = Visit 2 – Visit 1
Figure 8-1. A - Change in Serum 25(OH)D from Winter → Summer (n=84); B – Change in Serum 25(OH)D from Summer → Winter (n=43). Dark black line in both figures represents mean change in serum 25(OH)D between the two clinic visits.
Table 8-6a-d. Linear Mixed Models Regression Analysis for measures of insulin resistance and β-cell function

### Table 8-6a. Linear Mixed Models Regression Analysis for IS-OGTT

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<tr>
<th>Predictors</th>
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<th>P value</th>
<th></th>
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<th>P value</th>
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<td>Coefficient (95% CI)</td>
<td>P value</td>
<td>Coefficient (95% CI)</td>
<td>P value</td>
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</tr>
<tr>
<td>25(OH)D</td>
<td></td>
<td>0.008</td>
<td>(0.005, 0.011)</td>
<td>&lt;0.0001</td>
<td>0.005</td>
<td>(0.002, 0.008)</td>
<td>0.0009</td>
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<td>Seasongroup</td>
<td>Summer→winter</td>
<td>0.14</td>
<td>(-0.36, 0.64)</td>
<td>0.58</td>
<td>0.095</td>
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<tr>
<td></td>
<td>Winter→summer</td>
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<tr>
<td>25(OH)D*</td>
<td></td>
<td>-0.003</td>
<td>(-0.009, 0.002)</td>
<td>0.23</td>
<td>-0.003</td>
<td>(-0.008, 0.002)</td>
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<tr>
<td>Visit Number</td>
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<td>0.20</td>
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<td>0.19</td>
<td>(0.12, 0.26)</td>
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<td>Year 6</td>
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<tr>
<td>Vitamin D supplement use</td>
<td>No</td>
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<td>Physical activity</td>
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<td>0.00009</td>
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<tr>
<td>BMI</td>
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<td>-0.052</td>
<td>(-0.07, -0.04)</td>
<td>&lt;0.0001</td>
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### Table 8-6b. Linear Mixed Models Regression Analysis for HOMA-IR

<table>
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<th>P value</th>
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</thead>
<tbody>
<tr>
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<td>Coefficient (95% CI)</td>
<td>P value</td>
<td>Coefficient (95% CI)</td>
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<tr>
<td>25(OH)D</td>
<td></td>
<td>-0.008</td>
<td>(-0.01, -0.005)</td>
<td>&lt;0.0001</td>
<td>-0.005</td>
<td>(-0.01, -0.002)</td>
<td>0.0037</td>
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<td>Seasongroup</td>
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<td>-0.35</td>
<td>(-0.90, 0.20)</td>
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<td>-0.28</td>
<td>(-0.77, 0.21)</td>
<td>0.26</td>
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<td>Winter→summer</td>
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<td>25(OH)D*</td>
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<td>Visit Number</td>
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<tr>
<td>Vitamin D supplement use</td>
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### Table 8-6c. Linear Mixed Models Regression Analysis for IGI/IR

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<td>Coefficient (95% CI)</td>
<td>P value</td>
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<td>25(OH)D</td>
<td>0.005 (0.0001, 0.010)</td>
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<td>Season group</td>
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<tr>
<td>Summer → winter</td>
<td>0.45 (-0.31, 1.21)</td>
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<td>Winter → summer</td>
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<td>25(OH)D* Season group</td>
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<td>0.24 (0.11, 0.37)</td>
<td>0.0004</td>
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<td>Vitamin D supplement use</td>
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### Table 8-6d. Linear Mixed Models Regression Analysis for ISSI-2

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<td>Coefficient (95% CI)</td>
<td>P value</td>
</tr>
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<td>25(OH)D</td>
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<td>Summer → winter</td>
<td>0.25 (-0.09, 0.59)</td>
<td>0.15</td>
</tr>
<tr>
<td>Winter → summer</td>
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</tr>
<tr>
<td>25(OH)D* Season group</td>
<td>-0.003 (-0.007, 0.001)</td>
<td>0.15</td>
</tr>
<tr>
<td>Visit Number</td>
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</tr>
<tr>
<td>Year 3</td>
<td>0.15 (0.09, 0.21)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Vitamin D supplement use</td>
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<td>Physical activity</td>
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Chapter 9 : Overall Discussion

9.1 Summary

It is well recognized that the prevalence of type 2 diabetes is rapidly increasing and given the economic and social burden of type 2 diabetes and its complications, identifying risk factors and prevention strategies is crucial. Although a number of risk factors for insulin resistance, β-cell dysfunction and the metabolic syndrome have been identified, gaps still remain in understanding their etiology. Interest in the role of vitamin D in type 2 diabetes risk has been emerging, although gaps still exist in the literature given the use of suboptimal measures of insulin resistance and β-cell function, inadequate covariate adjustment and homogenous Caucasian study populations in some studies and also given very limited prospective data. The overall aim of this thesis was to examine the association of serum 25(OH)D with the two primary underlying disorders of type 2 diabetes, specifically insulin resistance and β-cell dysfunction. In addition, this thesis also included an investigation into the potential role of vitamin D in other risk factors for type 2 diabetes, particularly the metabolic syndrome and its components.

The objective for the first study of this thesis was to examine the cross-sectional association of serum 25(OH)D with measures of insulin resistance and β-cell function in the PROMISE cohort, which is comprised of a multi-ethnic population with risk factors for type 2 diabetes (Chapter 5). Overall, results indicated a significant inverse association of serum 25(OH)D with insulin resistance and a significant positive association of 25(OH)D with β-cell function after adjustment for sociodemographics, season, physical activity, PTH and BMI. Previous cross-sectional studies investigating the association of vitamin D with insulin resistance and β-cell function have reported inconsistent findings (2,23-26,29-34). In addition, most previous studies were conducted in largely Caucasian populations and often included suboptimal measures of insulin resistance and β-cell function. Therefore, the current study was valuable in that significant associations were found in a multi-ethnic Canadian population at risk of diabetes using more detailed validated measures of insulin resistance and β-cell function. Although potential effect modifiers including sex, ethnicity, BMI and season were investigated, a significant interaction of 25(OH)D with only BMI was found.
The magnitude of the association of 25(OH)D with both insulin resistance and β-cell function was modified by BMI, with a weaker association in those with a BMI ≥ 30 kg/m². Previous studies have also reported an attenuation of associations of 25(OH)D with diabetes/dysglycemia risk after adjusting for adiposity (17,31,32). Further, it has been consistently reported that individuals with greater body fat have lower levels of serum 25(OH)D. These findings may be due to the sequestering of 25(OH)D in adipose tissue (191,192), resulting in a weaker association between vitamin D and the outcome measures in obese individuals due to reduced bioavailability of 25(OH)D. However, volumetric dilution due to greater tissue mass, rather than sequestration, may better explain the low vitamin D status in obesity as cholecalciferol is stored in muscle and other tissues, in addition to adipose tissue (163,194,195). Additional research is clearly needed to investigate the mechanisms regarding the consistent inverse association of 25(OH)D and adiposity, and its potential effect on diabetes risk.

In addition to the consistent association of 25(OH)D with obesity, there has been a growing interest into the role of 25(OH)D with several other diabetes risk factors. In particular, the association of 25(OH)D with the metabolic syndrome has been gaining increased attention given that the metabolic syndrome represents a cluster of risk factors which increases risk of type 2 diabetes and cardiovascular disease, which are highly prevalent in the general population. Most previous studies examining the association of 25(OH)D with the metabolic syndrome had only assessed its association with the traditional components included in the well-recognized metabolic syndrome definitions (i.e. obesity, hypertension, dyslipidemia). In contrast, only a limited number of studies had investigated the association of 25(OH)D with the non-traditional components of the metabolic syndrome, including kidney dysfunction, non-alcoholic fatty liver disease, and inflammation. Emerging evidence suggests that these conditions are also part of the metabolic syndrome cluster and are predictive of type 2 diabetes risk (116,299,379,380). In this context, the primary objective of the second study was to assess the cross-sectional association of serum 25(OH)D with the metabolic syndrome and its traditional and non-traditional components (Chapter 6). Overall, higher 25(OH)D was significantly associated with a reduced presence of the metabolic syndrome. In addition, results indicated significant associations of 25(OH)D with various traditional and non-traditional components of the metabolic syndrome including inverse associations of
25(OH)D with triglyceride level, waist circumference, fasting insulin and alanine aminotransferase. A number of cross-sectional studies have examined the association of 25(OH)D with the metabolic syndrome, but inconsistent findings have been reported (38,39,41,43-47,50-55,57). Similarly, various individual components of the metabolic syndrome have been found to be significantly associated with low 25(OH)D in some, but not all studies (42,44,46,47,49,250-252). Previous studies have also reported a significant association of PTH with the metabolic syndrome, independent of 25(OH)D (39,51,52,249). This study, however, did not find a significant independent association of PTH with the metabolic syndrome, suggesting that PTH is likely not a potential mediator through which vitamin D is associated with the metabolic syndrome. Our current study was novel in that it included an examination of 25(OH)D with several non-traditional metabolic syndrome components in a large multi-ethnic cohort. The findings highlight the need for further investigation into the role of 25(OH)D with non-alcoholic fatty liver disease, dyslipidemia and inflammation.

Based on the findings of the first study described in Chapter 5, and given that much of the previous literature is limited by their cross-sectional design, a prospective study was then conducted to investigate the association of baseline 25(OH)D with insulin resistance and β-cell function after three years of follow-up in the PROMISE cohort. To date, there have been only two prospective studies conducted on this topic, both of which reported significant inverse associations of 25(OH)D with insulin resistance after 5 and 10 years of follow-up, respectively (11,58). No study had yet examined the prospective association of vitamin D with β-cell function. In the third study of this thesis, there was a significant positive association of baseline 25(OH)D with β-cell function and a significant inverse association of 25(OH)D with glucose homeostasis after 3-years of follow-up, but no significant association with insulin resistance. Given that it is generally recognized that β-cell dysfunction is a required disorder for the development of type 2 diabetes (72,119), the findings in the current study provide support for an important role of vitamin D in this chronic disease. However, the lack of a significant finding of baseline 25(OH)D with follow-up insulin resistance was surprising, given the results of the cross-sectional study in the PROMISE cohort (Chapter 5) and the results from the previous two prospective studies (11,58). However, it is possible that a longer follow-up of this cohort is needed to detect a significant prospective association of
25(OH)D with insulin resistance. In addition, PROMISE participants were more obese than those in the previous two prospective studies, and therefore it may be that the magnitude of obesity overwhelms the effect of 25(OH)D on insulin resistance in this study. The current study also found a borderline significant association of baseline 25(OH)D with incident diabetes after 3-years of follow-up. Again, it is possible that obesity was a stronger determinant of incident diabetes than 25(OH)D, as an initial significant association was attenuated to p=0.077 after adjustment for BMI. Alternatively, increased study power provided by a longer follow-up duration may have allowed for the detection of a greater significant association of baseline 25(OH)D with risk of developing diabetes. Although most previous prospective studies have reported a significant inverse association of 25(OH)D and diabetes risk, findings have not been consistent (8,11-13,17,18). In addition, all but one of these studies relied on a single baseline 25(OH)D measurement. In a recent prospective study, Pittas et al. (2012) investigated the association of 25(OH)D with risk of diabetes using multiple repeat measurements of 25(OH)D (13). They found that higher 25(OH)D (measured at baseline, 6-months and then annually up until 4 years) was significantly associated with a lower risk of diabetes after a mean follow-up of 2.7 years in the Diabetes Prevention Program. This study had greater power given that their sample size (n=2039) was much larger than in the PROMISE cohort. Given the limited number of prospective studies investigating the effect of 25(OH)D on insulin resistance and β-cell function, these study findings of a temporal association for vitamin D in the etiology of type 2 diabetes, contribute to the body of evidence supporting a role for vitamin D in type 2 diabetes.

The fourth study in this thesis focused specifically on the effect of seasonal variation in 25(OH)D on insulin resistance and β-cell function. A pilot study was designed with a particular focus on the effect of seasonal changes in 25(OH)D. The conception and design of this study was based on the hypothesis that seasonal fluctuations in 25(OH)D, which are often observed in those residing in northern latitudes and/or those with low environmental UVB exposure, may have adverse tissue-specific effects. In particular, Vieth (59) has hypothesized that such adverse effects of 25(OH)D are due to the lagged cellular adaptation of the vitamin D enzymes in response to seasonal fluctuations in 25(OH)D. This hypothesis regarding the behaviour of the vitamin D enzymes may be a potential explanation for the observed increased risks of prostate and pancreatic cancer with higher serum 25(OH)D levels.
in regions with low UVB (381,382). Given that little is known regarding the regulation of the paracrine metabolism of vitamin D, it remains uncertain whether fluctuations in 25(OH)D in areas with low year-round UVB exposure adversely affect various diseases and health conditions thought to be associated with 25(OH)D. Therefore, the primary objective for the last study of this thesis was to examine the effect of seasonal changes in 25(OH)D on insulin resistance and β-cell function in a subgroup of PROMISE participants (Chapter 8). Findings from this study did not indicate any significant effect of changes in 25(OH)D across seasons (winter to summer and summer to winter) on measures of insulin resistance and β-cell function over a mean 2.5 years of follow-up. However, the expected changes in 25(OH)D between seasons was not observed, particularly for those individuals with a first visit in the summer followed by a second visit in the winter, and may be due to the fact that clinic visits were approximately 2.5 years apart. Further, given our small sample size, additional studies with a larger study population and with repeat measurements made in consecutive seasons within the same year are needed.

Given the evidence supporting an association of vitamin D with the disorders underlying type 2 diabetes, several mechanisms have been proposed through which vitamin D may affect insulin resistance and β-cell function. First, vitamin D may directly enhance insulin action by increasing expression of insulin receptors (286) given the presence of the vitamin D response element in the promoter region of the insulin receptor gene (287). Vitamin D may also indirectly affect insulin action by regulating insulin-mediated intracellular processes via regulation of the calcium pool (288,289). With regards to β-cell function, vitamin D may exert direct effects through the binding of the circulating active form [1,25(OH)₂D] to the β-cell vitamin D receptor (294,295), and also through local calcitriol synthesis given that the 25(OH)D-1-α-hydroxylase enzyme has been shown to be expressed in β-cells (296). In addition, vitamin D may increase transcription of the insulin gene, given the presence of the vitamin D response element in the promoter region of this gene (287,297). Vitamin D may also indirectly influence β-cell function by regulating extracellular calcium and calcium flux through the β-cell. Another potential mechanism through which vitamin D may beneficially influence insulin resistance and β-cell function is by moderating the generation and effects of cytokines (264), given the strong association between type 2 diabetes and systemic
inflammation (299). Currently, however, the data are limited and inconsistent regarding the effects of vitamin D on systemic inflammation related to type 2 DM (264,300).

9.2 Strengths & Limitations

Interest in the role of vitamin D in type 2 diabetes has been rapidly increasing. At the time of commencing this thesis, there were a large number of cross-sectional studies examining the association of vitamin D with insulin resistance and β-cell function, although most studies were done in primarily Caucasian populations and many studies used suboptimal outcome measures. Strengths of this thesis work include the use of extensively validated measures of insulin resistance and β-cell function, calculated from fasting and post-challenge glucose and insulin samples during the oral glucose tolerance test. The PROMISE cohort provided a well-characterized study sample of individuals at risk of diabetes and low 25(OH)D levels given that these individuals all reside in Canada where endogenous vitamin D synthesis is absent during the winter months.

Some limitations of this thesis work also need to be highlighted. First, gold standard measures of insulin resistance and β-cell function were not used given that these procedures are time-consuming, burdensome, and expensive, and thus they are not ideal for use in large epidemiological studies. Second, BMI was used as a measure of adiposity. More accurate methods including bioelectrical impedance analysis (BIA) or dual-energy x-ray absorptiometry (DEXA) allow for true measures of body fat and its distribution. Therefore, more appropriate measures of fat mass (e.g. BIA), rather than BMI, to account for the effect of increased adipose tissue on serum 25(OH)D levels would have been beneficial. Further, Drincic et al. (2011) suggest that adjusting for obesity by using BMI may not adequately account for the effect of 25(OH)D with body size (194). Another limitation, which was specific to our prospective study (Chapter 7), was that only a single baseline measurement of 25(OH)D was made, which may not adequately reflect long term vitamin D status. Although it has been suggested that a single measure of 25(OH)D is reflective of levels over time (383), given the recent interest and increased media attention on the beneficial effects of vitamin D and therefore concomitant increases in vitamin D consumption in the general population, repeat measurements of 25(OH)D are required to adequately capture vitamin D
status over time, resulting from changes in lifestyle (diet, supplement use, sun exposure) and weight. Although serum 25(OH)D is the best measure of vitamin D nutritional status, it would have been beneficial to also have included an assessment of dietary intake and sun exposure behaviours for PROMISE participants at all clinic visits. However, baseline PROMISE visits had already been completed upon commencement of this thesis and the 3-year follow-up visits were well underway. Lastly, given that all studies in this thesis work were observational in nature, the issue of residual confounding is present despite multivariate adjustment, which relates to the possible imprecise measurement of included confounders such as physical activity or supplement use or due to unmeasured potential confounders. In addition, the observational design of the studies included in this thesis do not allow one to adequately assess whether the association of low 25(OH)D with type 2 diabetes risk is causal or potentially an epiphenomenon. However, observational studies are important in the field of health research as they allow for phenomena to be examined in a real world setting and also provide necessary evidence and justification for clinical trials to be conducted.

9.3 Future Directions

The studies comprising this thesis have extended the scientific literature regarding the association of vitamin D with metabolic disorders underlying type 2 diabetes. In particular, it is important to note that most previous studies have been cross-sectional in nature, and there are currently very limited prospective data on this specific topic. Therefore, additional longitudinal studies are needed to examine the association of serum 25(OH)D with the progression of insulin resistance, β-cell function, and the metabolic syndrome over time. Observational studies, particularly longitudinal studies, help to establish the time-course and natural history of exposure-outcome associations, define exposure-response relationships across a wide range of exposure, identify genetic susceptibility and other potential effect modifiers and also identify critical subgroups for intervention. In particular, future studies should attempt to include multi-ethnic populations which allow for increased generalizability and also allow for testing of potential ethnic-specific effect modifiers. Further, studies should also include repeat measurements of 25(OH)D over time to adequately characterize the role of long term 25(OH)D status. Therefore, continued epidemiological investigation into the
role of vitamin D in diabetes risk is warranted, as these studies shed light on critical methodological aspects related to the design of good quality randomized controlled trials.

9.4 Conclusion

Diabetes and its associated complications are significant public health problems. This thesis found that lower baseline serum 25(OH)D was cross-sectionally related to greater insulin resistance, poorer β-cell function and a higher prevalence of the metabolic syndrome, and prospectively related to poorer β-cell function at the 3-year follow-up. In addition, seasonal variation in 25(OH)D levels did not have a significant effect on measures of insulin resistance and β-cell function longitudinally. Overall, these findings suggest a potential beneficial role of vitamin D in diabetes risk. Confirmation of these findings in future observational studies and, specifically, well-designed randomized controlled trials potentially has significant public health implications given the high prevalence of suboptimal vitamin D status in the general population and the relative ease and low cost at which such an intervention may be implemented.
Chapter 10: References


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